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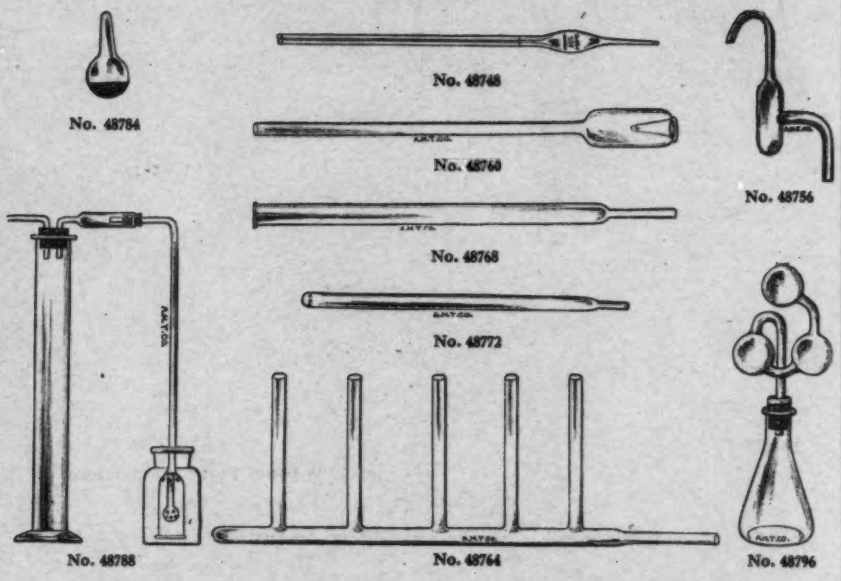

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 41 OCTOBER 1, 1916 No. 4

THE RELATION OF ACID TO GASTRIC DISCHARGE AND DUODENAL REGURGITATION IN THE DOG

W. E. MORSE

From the Laboratory of Physiology of the Northwestern University Medical School

Received for publication July 12, 1916

From a review of physiological literature one receives the impression that the presence of an acid reaction in the stomach is essential to and hastens gastric discharge. This is not entirely concordant with clinical observations. In cases of so-called hyperacidity a retarded emptying of the stomach with resulting atony and dilatation is often found. Also in cases of achylia gastrica, where there is practically no free acid found in the stomach, the normal rate of discharge is often unimpaired. It therefore seems probable that the presence of an acid reaction is not the only essential factor in the control of gastric discharge.

Hirsh (1) has reported that solutions of inorganic acids leave the stomach slowly and inferred that this slow exit was due to the stimulating effect of the acid on the mucosa of the duodenum. Cannon (2) in his fluoroscopic work on cats, fed mashed potato and bismuth, and observed that hastening the appearance of an acid reaction hastened the initial gastric discharge. Hedblom and Cannon (3) fed acidified mashed potato and bismuth to their animals and by fluoroscopic observations estimated that the rate of discharge was increased over the normal rate up to an acidity of 0.25 per cent, acidity above 0.25 per cent reduced the rate of discharge to or below the normal. Heidenhain (4), Pawlow (5), Sommerfeld (6), Carlson (7), and others have demonstrated an acidity of 0.4 to 0.5 per cent in the pure gastric juice of animals and man. Boldyreff (8), has presented evidence that this high acidity is neutralized to an optimum acidity of 0.15 to 0.2 per cent by regurgitation of duodenal juices of which the pancreatic juice

is most important. Through a gastric fistula in dogs, Boldyreff injected acidified fluids into the stomach and found that the acidity was rapidly lowered to 0.15 to 0.2 per cent and the rate of discharge was increased with the reductions of the acidity. After ligating the pancreatic ducts he found that the reduction of the acidity and the rate of discharge were greatly retarded. Recently Spencer, Meyer, Rehfuss and Hawk (9), have demonstrated the presence of pancreatic trypsin in the gastric contents, the amount present being greater with low acidity than with high acidity. In the same article these investigators noted that sodium bicarbonate in 1 per cent solutions left the stomach rapidly while 5 per cent left slowly. By means of the X-ray Tabora (10) studied gastric peristalsis and motility in normal individuals. He used bismuth alone and combined with hydrochloric acid. When acid was used the peristalsis was deeper and more energetic but the emptying time was 50 per cent longer. Carlson (11) found that the rhythms of the empty stomach appear whether the mucosa is alkaline or acid, but are inhibited by strong acidity or alkalinity. The inhibition was greater with 0.5 per cent hydrochloric acid than with a similar concentration of sodium carbonate. Clinically considerable variation in the relation of acidity to discharge is also found in observations made upon patients with so-called hyperacidity. Osler (12) finds that most cases of hyperacidity show a normal or increased motility although in 30 per cent of his series there was a coincident atony of the stomach with some retention. Strümpell (13) usually finds the stomach empty within three to four hours after a test meal, which he attributes to increased motility and an increased digestive power of the gastric juice. Reed (14) maintains that hyperacidity and some degree of gastric hyperesthesia which frequently is coincident, are the chief causes of the common motor disturbances such as spasm of the cardia and of the pylorus with long delayed emptying of the stomach. Kemp (15) states that motility is unimpaired in cases of hyperacidity, unless complicated with atony and insufficiency which it often produces. Krehl (15) considers hyperacid secretions with resulting pylorospasm as an etiological factor in gastric dilatation.

From the above noted somewhat conflicting experimental and clinical observations it is evident that the relation of gastric acidity to motility, discharge, and duodenal regurgitation is still sufficiently problematic to justify further investigation under varying conditions. The conditions imposed in this research were first; a normal fasting animal stomach, second, fluid injections of constant temperature and

quantity but of varying acidities; third, abolishment of inhibiting sympathetic reflexes by pithing the spinal cord.

TECHNIQUE

Dogs were used throughout the experiments. The animals were unfed for forty-eight hours to secure total absence of food from the stomach. This however was not obtained in every case, as noted below, owing to the indigestible nature of some of the material previously ingested. Light ether anaesthesia was maintained by the tracheal cannula-ether bottle method. Through a laminectomy incision the spinal cord was destroyed from the sixth to the tenth dorsal segments. This destruction of the cord severed the splanchnics from their connection with the central nervous system and thus eliminated inhibitory reflexes. Cannon (17) determined that section of the splanchnics in cats resulted in no change of the normal movements of any part of the alimentary canal. Carlson (18) found that section of the splanchnics in dogs increased the gastric tonus and augmented the gastric hunger contractions. It is therefore evident that destruction of the inhibitory mechanism does not diminish gastrointestinal activity. Fluids were introduced and withdrawn from the stomach by means of a glass tube inserted through an esophageal fistula in the cervical region. The fistula was made to avoid laryngeal reflexes and prevent swallowing of saliva. The portion of the tube used for insertion into the stomach was bent at an angle of 10 degrees 1 inch from its termination and provided with a rubber extension. The protruding portion was also bent in the same plane as an index to the direction assumed by the bent portion of the inserted end. The protruding end was connected with a funnel for introduction of fluids. When aspirating the stomach contents a suction bulb was substituted for the funnel. By placing the animal on its left side and directing the bend in the tube toward the animal's left, the most dependent portion of the fundus of the stomach could be reached and completely aspirated.

At the beginning of each experiment, after removing the fluid contents of the stomach, our aspirating technique was tested and the stomach lavaged by the introduction and immediate withdrawal of 200 cc. of water. If 198 to 200 cc. were recovered the method was considered efficient, 2 cc. being allowed for immediate discharge through the pylorus. If less than 198 cc. were recovered the tube was readjusted and the test repeated until satisfactory results were obtained. It was

seldom found necessary to repeat the test. By carefully measuring the amount of fluid introduced into the stomach and the amount withdrawn, the amount of discharge or of duodenal regurgitation may be determined. The question immediately arises however: What effect has the introduction of water and various acid solutions on the rate of *gastric secretion?* This secretion, if considerable, would be a disturbing factor in the results. Pawlow (19) found that in dogs gastric secretion is slightly stimulated by the introduction of 500 cc. of water into the stomach. The introduction of 100 to 150 cc. of water produced no secretion in 50 per cent of his cases, the secretion in the case of 500 cc. of water being due to the long continued contact of the water with the gastric mucosa. Solutions of meat ash, chloride and hydrochlorie acid produced exactly the same effect as water alone. It was therefore considered probable that the amount of secretion produced when 200 cc. of water or hydrochloric acid solutions are introduced is small and not a disturbing factor in the comparative values of discharge or duodenal regurgitation. In order to determine positively that secretion is not a disturbing factor under the conditions of this investigation, a separate series of experiments was made upon six animals using the same technique but with the pylorus ligated. The results of these experiments usually showed no secretion, and when any occurred it was so small in amount as to be negligible. This series will be extended and reported in detail. When more fluid was recovered than was introduced the increase was attributed to duodenal regurgitation as gastric secretion or hemorrhage could not be considered sufficient to produce the results.

In the above noted technique the stomach and intestines were not traumatized. The abdomen was not opened until the end of the experiment when an autopsy was made and the gastric contents noted as a further check upon the aspirating efficiency. Previous to the adoption of this technique two other methods were tested. First the rate of gastric discharge was determined by recovering the discharged fluid from the duodenum through a fistula. Second a tube was introduced into the stomach through an incision in the stomach wall and fluids were introduced and withdrawn the discharge being noted by difference. Both of these methods were discarded because of local inhibitions produced.

Data were obtained from two series of experiments. In the first fourteen animals were used. Solutions of acidity varying from 0 to 0.5 per cent hydrochloric acid were introduced into the stomach.

The quantity used was uniformly 200 cc. and the temperature 37°C. Water was first injected. The acidity was increased 0.1 per cent with each injection until 0.5 per cent hydrochloric acid was reached. Each injection was aspirated after an interval of thirty minutes. The results from two of the animals of this series were discarded as unreliable. In the first case aggravated retching produced regurgitation with loss of considerable fluid thus destroying the accuracy of the measurements. In the second case 25 cc. of fluid and some solid material were found in the stomach at autopsy.

In the second series four animals were used. The time interval was shortened to twenty minutes and the acidity of the solutions was increased 0.2 per cent with each injection until 1 per cent hydrochloric acid was reached.

The post mortem examination of the stomach contents as a check upon the aspirating technique was usually gratifying, In six animals of the first series the stomach was empty, in two there was a small amount of solid material, such as bone and remnants of potato but no fluid; in one there was 25 grams of solid material and 4 cc. of fluid. In the remaining three animals of this series a small amount of thick discolored mucus was found. In the second series two stomachs were empty, one contained some mucus and one some solid material but no fluid.

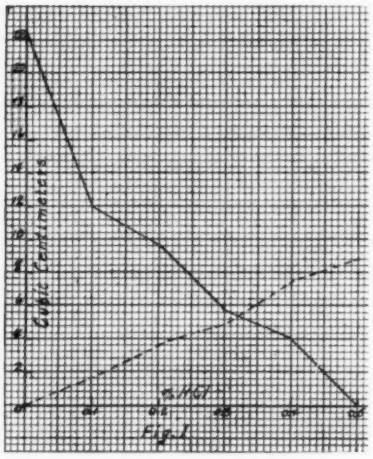
Chart I

DOG NO.	H_2O		HCl—0.1%		HCl—0.2%		HCl—0.3%		HCl—0.4%		HCl—0.5%	
	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.
1	20	0	25	0	0	10*	5	0	0	0	0	0
2	27	0	27	0	0	18*	0	20	20	0	0	25
3	5	0	0	0	25	0	5	0	0	18*	0	0
4	50	0	25	0	35	0	15	0	0	5*	0	25
5	20	0	5	0	10	0	10	0	0	15*	0	5
6	5	0	0	0	0	15*	0	0	0	30	0	10
7	55	0	50	0	5	0	0	7*	0	3	0	0
8	55	0	0	20*	10	0	10	0	20	0	0	20
9	5	0	0	0	7	0	5	0	0	0	0	10*
10	10	0	0	0	20	0	0	30*	0	0	0	10
11	10	0	5	0	0	0	10	0	10	0	0	5*
12	10	0	5	0	0	0	10	0	0	10*	0	5
Average.....	$22\frac{1}{4}$	0	$11\frac{10}{12}$	$1\frac{8}{12}$	$9\frac{1}{3}$	$3\frac{7}{12}$	$5\frac{5}{8}$	$4\frac{3}{4}$	$4\frac{1}{6}$	$7\frac{1}{2}$	0	$8\frac{3}{4}$

D, discharge from stomach; R, regurgitation into stomach; *, first appearance of regurgitation.

RESULTS

The results of the first series as tabulated in Chart I show a general uniformity although certain deviations are noted. In eight of the twelve animals the greatest discharge was obtained with water. The average discharge with water was 22.2 cc., which is less than that obtained by Moritz (20) from duodenal fistulae. The average discharge for the various dilutions decreased with increase of acidity



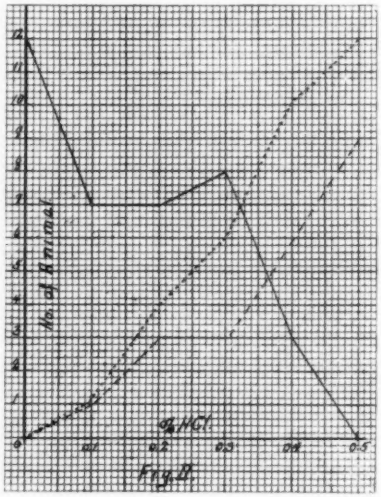


Fig. 1. Based on 12 experiments, ——— average discharge; - - - - - average regurgitation.

Fig. 2. Based on 12 experiments. ——— Number of animals showing discharge. - - - - - Number of animals showing regurgitation. ..... Number of animals having shown regurgitation at some previous stage of the experiment.

(fig. 1). These findings are in accord with Hirsch's (1) finding that inorganic acids leave the stomach slowly.

Referring to Chart I the following individual deviations are noted. Dogs 1, 3, 9 and 10 have slightly greater discharge with acid solutions than with water. Dogs 2, 3, 4, and 8 have at some point a greater discharge with acid solutions than with some preceding lesser acidity but not greater than with water.

In addition to the progressive diminution in the amount of discharge there is an increase in the average amount and in the frequency of regurgitation with increase in the acidity (figs. 1 and 2). In every case regurgitation occurred at or before 0.5 per cent acid was reached. Hicks and Fisher (21) recently reported regurgitation in 38 per cent of 56 trials on dogs when 0.5 per cent hydrochloric acid was introduced into the stomach. After section of the splanchnics they obtained regurgitation in all of nine trials. Change in color was considered sufficient evidence of regurgitation by these investigators after they found that one drop of bile in 100 cc. of water produced a bright straw color. In our series of experiments regurgitation was evidenced by the increase of the fluid content of the stomach. The appearance of bile coloring in the aspirated fluid was occasionally noted but if constantly present was usually obscured by slight hemorrhage which occurred between 0.3 and 0.9 per cent acidity. A control color test with blood and hydrochloric acid solutions showed that from 1 to 3 cubic centimeters of blood in the aspirated contents would produce the color change and hence hemorrhage was not sufficient to account for the increase of the gastric contents. The mucosa at autopsy invariably showed excoriated areas more numerous in the fundus than in the antrum.

In every case some discharge was obtained with water. Occasionally when using acidified fluid the amount aspirated was the same as that introduced thus giving us no evidence of discharge or regurgitation. The number of animals showing discharge decreased with increase of acidity up to 0.5 per cent acid, where a discharge was never obtained (fig. 2).

Chart II

DOG NO.	H_2O		HCl—0.2%		HCl—0.4%		HCl—0.6%		HCl—0.8%		HCl—1.0%	
	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.
1	10	0	0	0	5	0	0	5*	0	10	0	20
2	0	0	0	0	0	0	0	5*	0	15	0	50
3	60	0	0	20*	0	5	0	30	0	5	0	5
4	8	0	0	0	0	0	0	0	0	0	0	10*
Average.....	19½	0	0	5	1¼	1¼	0	12¼	0	7½	0	21½

D, discharge from stomach; R, regurgitation into stomach; *, first appearance of regurgitation.

The results of the second series (Chart II) with higher percentages of acid in general corroborate the findings of the first series. In only one case did any discharge occur except with water. Dog No. 1 shows 5 cc. discharge with 0.4 per cent acid, but this is 6 cc. less than that obtained with water.

DISCUSSION

The most prominent feature of the results, as tabulated above, is the progressive diminution in the rapidity of discharge with increase in the acidity. This does not concur with the findings of Hedblom and Cannon (3) in which the optimum acidity for maximum discharge was found to be 0.25 per cent. However Hedblom and Cannon's work was done on cats fed mashed potato and bismuth. At autopsy of their animals they found the material fed was separated into masses in the intestines (22). Possibly the difference between their results and those herein reported may be explained by the fact that only the surface of the semi-fluid material passing through the pylorus was presented to the duodenal mucosa. In this diffusion would be retarded and the acidity of the surface could be quickly neutralized by the alkaline duodenal juices without exciting inhibition of discharge or regurgitation. This process could continue as the peristaltic action of the duodenum brought new surfaces of the material in apposition with the mucosa. But in the case of acidified fluid, the whole acid content is in effect at once presented to the mucosa and the neutralizing power of the duodenal juices is exhausted before the volume of fluid admitted is neutralized below the point sufficient to produce inhibition of discharge or regurgitation.

Boldyreff (8) finds the optimum acidity to be 0.15 to 0.2 per cent. He believes that the constant acidity of 0.4 to 0.5 per cent of the gastric juice is reduced by influx of duodenal juices. This view is supported by the presence of trypsin in the gastric contents as demonstrated by Spencer, Meyer, Rehfuss and Hawk (9). The constant occurrence of regurgitation as noted in our experiments may be taken as further evidence in support of Boldyreff's theory. This regurgitation always occurred at 0.5 per cent acid or before 0.5 per cent acid was reached and the average amount of regurgitation as evidenced by the quantitative estimation was directly proportional to the acidity of the fluid in the stomach.

Throughout these experiments the presence of acid in the stomach in general has been observed to retard rather than hasten gastric dis-

charge. As noted above Spencer, Meyer, Rehfuss and Hawk (9) observed that 1 per cent sodium bicarbonate leaves the stomach rapidly, while 5 per cent leaves slowly. In the light of their observations and the results of this series of experiments it appears that the acidity of fluid gastric contents has no function in producing or hastening gastric discharge, but the discharge seems to be controlled by the irritability or non-irritability of gastric contents to the duodenal mucosa.

The optimum acidity as reported by different investigators and as observed in these experiments seems to vary with the physical condition of the material used and is lower for the more fluid content. The optimum acidity seems to be that which can be quickly neutralized or diluted to a non-irritating point without exciting inhibition of discharge or regurgitation.

SUMMARY

1. Water is discharged from the fasting stomach of anesthetized pithed dogs more rapidly than any percentage of acid.

2. The rate of discharge is decreased with increase of acidity.

3. Duodenal regurgitation, as evidenced by increase in the contents of the stomach, often occurred at 0.2 per cent acidity and in nearly all trials occurred at or before 0.3 per cent acidity was reached.

4. Increase in acidity increases the frequency and amount of regurgitation from the duodenum.

I wish to express my gratitude to Dr. R. G. Hoskins and Mr. R. E. Lee Gunning for their many valuable suggestions, and assistance throughout the work.

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STUDIES ON BIOLUMINESCENCE[1]

II. On the Presence of Luciferin in Luminous Bacteria

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Princeton University

Received for publication, July 15, 1916

In a recent issue of Science (1) I pointed out that Dubois (2) had demonstrated in 1885 the existence of two substances which are concerned in the light production of the West Indian Cucullo, *Pyrophorus noctilucans*, and also of the boring mollusc, *Pholas dactylus*, one, thermostabile, called luciferin; and another thermolabile, called luciferase and having all the properties of an enzyme. Dubois demonstrated this by filling two test tubes with an extract (luminous) of the luminous organs, then boiling one tube, which destroys the luciferase but not the luciferin, and allowing the other tube to stand until the light disappears, i.e., until all the luciferin is completely oxidized in the presence of oxygen. If the tube contents (both dark) are mixed, light appears. Luciferin and luciferase have now been brought together in presence of oxygen and the light lasts until the remaining luciferin is completely oxidized. I have been able to verify the above for *Pyrophorus* and to show that the common American fire-flies contain luciferin and luciferase; also to demonstrate that luciferin from one genus of fire-fly (*Photinus*) will act with luciferase from another genus (*Photuris*) and vice versa; also that fire-fly (*Lampyridae*) luciferase will act with *Pyrophorus* (*Elateridae*) luciferin and vice versa. Non-luminous parts of fire-flies contain no luciferase, however, nor do non-luminous insects or extracts of earth-worms, slugs or pill bugs (3).

Oxygen is essential for light production by luciferin and luciferase, but if oxygen is absent the luciferase is able to change the luciferin in some way. This may be shown by allowing an oxygen-free aqueous

[1] The first paper of this series entitled, Studies on Light Production by Luminous Bacteria appeared in this Journal, 1915, xxxvii, 230. The third paper follows the present one. Papers upon the chemistry of luminescence in the fire-fly and in luminous marine forms will follow.

mixture of luciferin and luciferase to stand. If oxygen is admitted in five minutes we obtain light but if we wait for one hour before admitting oxygen, no light is produced (4). Luciferin alone or luciferase alone in solution are stable for over six hours.

Luminous bacteria contain luciferin also but in very small quantities as we might expect from the intensity of the light which is far less brilliant than the fire-fly although produced continuously. I have been wholly unable to demonstrate it by a repetition of Dubois' experiment, due in part I think to the fact that in bacteria the luciferase is an endoenzyme which cannot be extracted by the ordinary biochemical methods. If we use the luciferase of the fire-fly we can obtain light from a bacterial luciferin prepared in the following way: by adding absolute alcohol to a dense mass of the bacteria, then removing the alcohol by centrifuging and quickly drying by evaporation in vacuo. The resultant powder gives no light with water but does phosphoresce very faintly with a solution of fire-fly luciferase. Similar material prepared with acetone instead of alcohol gave no light with fire-fly luciferase, nor did bacteria heated from one-half to two minutes to 100°C. Thinking that bacterial luciferin as well as the luciferase might be destroyed below 100°, I tried heating to lower temperatures, 90°, 80°, 70°, 60° and 50° for two minutes, but in no case was light produced when fire-fly luciferase was added to these bacteria. Probably the amount of luciferin in luminous bacteria is so small that it is burned up before the temperature rises sufficiently to destroy the luciferase, whereas, addition of alcohol instantly prevents the action of the luciferase. In order to obtain a bacterial powder which will give light with fire-fly luciferase it is necessary to remove the alcohol and dry as quickly as possible; otherwise the luciferin is also destroyed.

Addition of ether to a mass of luminous bacteria causes the light to disappear rapidly and I thought that the luciferase might be destroyed and not the luciferin, but bacteria so treated, after the ether had been evaporated by a current of air, gave no light with fire-fly luciferase. Negative results were obtained also with an alkaline ($\frac{M}{12}$ KOH) extract of luminous bacteria neutralized with HCl. A precipitate of nucleoprotein is produced on neutralization. Apparently the bacterial luciferin is formed in such small quantities from its precursor or is so unstable that all methods of obtaining it have failed with the exception of the rapid precipitation by alcohol.

That bacterial luciferin, like fire-fly luciferin, is changed and disappears on standing in contact with *oxygen-free* water, was demonstrated

by my earlier attempts to extract a photogenic substance with oxygen-free solvents (5).

It may be pointed out in passing that the experiments with bacterial luciferin were made with an unfiltered suspension of the bacteria since they are small enough to pass filter paper. Fire-fly luciferin may be obtained in a perfectly clear filtrate free of cells and cell fragments.

On the other hand, I have found it impossible to obtain a bacterial luciferase. Neither bacterial luciferin nor fire-fly luciferin will react with any sort of bacterial preparation which might be supposed to contain luciferase. I have tried extraction with water and chloroform water of both the fresh bacteria and those rapidly dried and then ground with sand. The methods of growing, collecting and drying the bacteria are described in my earlier paper (6). It is not surprising that this is so, for many enzymes are found in bacteria in a condition which defies extraction except by means of the Büchner press and it is difficult to obtain luminous bacteria in sufficient quantity for this method. I feel certain that the oxidases are contained in these bacteria in this endoenzyme condition. All my efforts to obtain an oxidase in solution which will oxidize the common oxidase reagents; guaiac a-napthol, para-phenylen-diamine, a mixture of a-napthol and para-phenylen-diamine, phenolphthalin and pyrogallol have failed, even though hydrogen peroxide is added.

Similar results have been obtained by other workers. Gessard (7) showed that a melanogenic variety of *Bacterium pyocyaneum* would turn tyrosin brown but he was unable to separate a solution of tyrosinase from the bacteria. Lehman and Sano (8) found also that *Actinomyces chromogenes, Bacterium putidum* and *B. phosphorescens* would oxidize tyrosin in the culture medium but *Vibrio indicus*, which phosphoresces strongly, *Sarcino lutea, B. typhi, B. coli*, and many others would not. They found in addition that a substance oxidizing aloin and giving a very weak guaiac reaction could be extracted from *Actinomyces chromogenes, B. putidum and B. phosphorescens* by a mixture of two parts glycerine to one of water, but no tyrosin reaction could be obtained with this extract. Here also the tyrosinase is apparently in an endoenzyme condition.

Although luciferase is no doubt an oxidase it is not like the oxidases of plant juices, of potato or turnip juice for example. Fire-fly or bacterial luciferin and turnip juice or potato juice will give no light when mixed even upon the addition of H_2O_2. Fire-fly luciferase is also very

readily destroyed by chloroform water while the oxidase of turnip juice can be preserved indefinitely by chloroform. Fire-fly luciferin is not destroyed by chloroform but a solution of luciferin preserved under chloroform loses its power to phosphoresce in the course of ten to twenty days. In many ways the luciferin of the fire-fly is much the more stable substance. A complete account of its properties will be found in a subsequent paper of this series.

SUMMARY

1. Attention is called to the discovery of R. Dubois in 1885–1886 of the presence of a thermostabile, oxidizable substance, *luciferin*, and a thermolabile enzyme-like substance, *luciferase*, in the luminous organs of the mollusc, *Pholas dactylus* and the beetle, *Pyrophorus noctilucans*.

2. Similar substances occur in the American fire-flies and can be obtained in solution filtered through filter paper.

3. Luciferin from *Photinus*, *Photuris*, or *Pyrophorus* will give light with luciferase from any of the above three genera or vice versa.

4. Non-luminous parts of fire-flies or extracts of many other non-luminous insects, slugs, earth-worms, or pill bugs give no light with luciferin.

5. Luciferin in a very impure state can be obtained by precipitating luminous bacteria with alcohol and will give light with fire-fly luciferase.

6. Luciferase cannot be obtained from luminous bacteria because it is probably present as an endoenzyme.

7. Oxidases for guaiac, a-napthol, para-phenylen-diamine, phenol, phenolphthalin, pyrogallol, or indo-phenol formers, if present, are also in an endoenzyme condition.

8. In presence of oxygen, luciferase decomposes luciferin with light production; in absence of oxygen the luciferin is also decomposed but without light production.

9. Luciferase (fire-fly) is quickly destroyed by ether and chloroform, in this respect differing from the vegetable oxidases. Luciferin (fire-fly) is not readily destroyed by ether or chloroform.

10. Luciferin of bacteria or the fire-fly will give no light with the oxidases of potato juice either with or without addition of hydrogen peroxide.

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STUDIES ON BIOLUMINESCENCE

III. On the Production of Light by Certain Substances in the Presence of Oxidases

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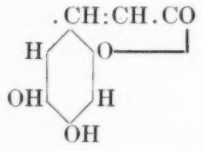
Since the researches of Radziszewski in 1877 (1) and 1880 (2) on lophin and various oils, alcohols and aldehydes we have come to recognize an ever increasing number of substances which will luminesce under appropriate conditions at temperatures approaching those compatible with life. Lophin (triphenylglyoxaline) gives an especially good light with alcoholic potash at 60°C., as do many essential oils. Trautz (3) has added additional substances to the list of chemiluminescent reactions, as has also R. Dubois (4), who discovered the luminescence of aesculin, a glucoside obtained from horse chestnut bark and chemically a combination of glucose and aesculetin.



These substances all require fairly strong alkali, although I have a sample of Bergamot Oil which gives a beautiful light when mixed with n/10 NaOH at 20°C. and a faint light at 5°C. At 20°C. a faint light appears with n/80 NaOH. On warming the light is brighter. n/80 NaOH is, however, incompatible with life. Lophin and aesculin will not luminesce except with alcoholic NaOH or KOH.

Ville and Derrien (5) made a step forward when they showed that lophin would luminesce with blood and H_2O_2 and Dubois (6) has recently discovered that aesculin also will give light with blood and H_2O_2. The blood contains an oxygen carrier, haemoglobin, which ac-

celerates the oxidation of the lophin and aesculin. I can confirm the statement of Dubois, and of Ville and Derrien and find also, contrary to Dubois, that aesculin will give light with $K_2Mn_2O_8$ and H_2O_2. It is best to keep the temperature about 60°C. I found also that aesculin would give light with $FeCl_3 + H_2O_2$ but not with $FeCl_3$, or $K_2Mn_2O_8$ or H_2O_2 alone, nor with plant extracts rich in oxidases (turnip, potato and horse radish root) either with or without H_2O_2, and that blood extract still retains its power to produce light with lophin after boiling. Blood will oxidize guaiac after boiling and upon addition of H_2O_2. Oxonized turpentine can take the place of H_2O_2 in oxidation of aesculin.

It is of interest to note the similarity between the oxidation with light production of aesculin or lophin by means of the blood oxidases, and the oxidation of luciferin by luciferase, the substances found in luminous animals. Except that luciferase is instantly destroyed by boiling and is active at very low temperatures the parallel is indeed striking.

I have recently discovered (7) a luminescent reaction which mimics in all details the process occurring in luminous organisms and shows us the essential nature of that process. *The oxidation of a mixture of pyrogallol* + H_2O_2 *by the vegetable oxidases occurs with the production of light.* The reaction is highly interesting and remarkable for the following reasons. Perceptible light is produced with the concentration of pyrogallol m/32000 i.e., 1 part in 254,000 parts of solution; a faint light is produced at 0°C. and a bright light at 10°C.; KCN inhibits the reaction in m/2000 concentration; boiling destroys the oxidase just as it does luciferase.

The pyrogallol + H_2O_2 corresponds to luciferin and the vegetable oxidase to luciferase. The light produced is yellowish white and lasts two to four minutes with an intensity about equal to that of a suspension of luminous bacteria. The pyrogallol is converted into colored compounds by means of the oxygen of H_2O_2 which disappears in the process. As we shall see later, the oxidase is also used up in the reaction so that it does not function as a true enzyme. Whether luciferase is used up in oxidizing luciferin is unknown at present but I expect to present data on this subject in a subsequent paper. Hydrogen peroxide is necessary for light production even when potato juice is used as the oxidase solution, i.e., even when a juice containing an oxidase which will oxidize guaiac tincture directly (without addition

of H_2O_2) is used. It is then strictly a peroxidase which is responsible.[1] Blood also gives the reaction. Catalase takes no part and may be destroyed by heating to 60°.

In studying this luminescent reaction more closely, I have used mostly pure potato juice (i.e., the pressed, unfiltered extract of grated potatoes, strained through cheesecloth and decanted from the starch grains) or a 1 per cent ox blood extract (i.e., 1 gram of dried defibrinated ox blood to 99 parts water) added to an equal volume of a half and half mixture of m/100 pyrogallol and 3 per cent H_2O_2 (Merck's Blue Label). The experiments will be given in the form of tables or data under the following heads:

1. Concentration of the three substances necessary for light production.
2. Substances taking the place of blood or potato oxidase, pyrogallol and H_2O_2.
3. Effect of temperature on the reaction.
4. Effect of KCN, NaOH and HCL.
5. Effect of fat solvent anaesthetics.
6. Is the oxidase a catalyzer?

1. Concentration of the three substances necessary for light production. (Tables 1, 2 and 3).

In general the more concentrated the blood or potato juice the brighter the light, and the more concentrated the pyrogallol (to a limit) the brighter the light, and the longer it lasts. Too strong pyrogallol precipitates the blood extract and potato juice, the former in weaker concentration than the latter (see table 1). In about m/8000 concentration the pyrogallol gives a bright light with potato juice and increasing the concentration above this does not increase perceptibly the brightness.

[1] The difference between potato juice, which oxidizes guaiac without addition of H_2O_2, and turnip juice, which requires the addition of H_2O_2, appears to lie in the fact that the potato juice contains a substance which oxidizes spontaneously in presence of oxygen to a peroxide, while the turnip juice does not. Consequently, we must add a peroxide to the turnip juice. This spontaneously oxidizable substance was called by Bach and Chodat oxygenase. Both juices contain peroxidase. The "direct oxidases" (of potato) consist, then, of peroxide (oxygenase) + peroxidase, while the "indirect oxidases" (of turnip) are peroxidase alone. In this paper oxidase is used as a general name for an oxidizing enzyme and peroxidase for an enzyme transferring oxygen from a peroxide to an oxidizable substance. See the account of this subject in Bayliss' Principles of General Physiol., 1915, 584.

TABLE 1

CONCENTRATION OF PYROGALLOL IN A MIXTURE CONTAINING (AFTER MIXING) 50 PER CENT POTATO JUICE AND 0.93 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	CONCENTRATION OF PYROGALLOL IN A MIXTURE CONTAINING (AFTER MIXING) 0.93 PER CENT H_2O_2 AND 0.5 PER CENT BLOOD EXTRACT	CHARACTER OF LIGHT FROM THE MIXTURE
m/4	Faint	m/40	Faint
m/8	Brighter	m/80 to m/8000	Bright
m/16 to m/8192	Bright	m/16000	Fair
m/16384	Fair-faint	m/32000	Faint
m/32768	Very faint	m/64000	Very faint—none
m/65536	None	m/128000	None

TABLE 2

CONCENTRATION OF POTATO JUICE IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 0.75 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC	CONCENTRATION OF BLOOD EXTRACT IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 0.75 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC
per cent			*per cent*		
25	Bright	Blue	0.5	Bright	Blue
10	Faint	Blue	0.25	Faint	Blue
5	Very faint	Faint blue	0.05	Very faint	Blue
2.5	None	Faint blue	0.025	None	Blue
0.5	None	Very faint blue	0.01	None	Blue

TABLE 3

CONCENTRATION OF MERCK'S SPECIAL H_2O_2 IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 50 PER CENT POTATO JUICE	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC
per cent		
0.47	Bright	Blue
0.23	Fair	Blue
0.11	Weak	Blue
0.05	Faint	Blue
0.025	Very faint	Blue
0.012	None	Blue

Each substance must be present in a definite small concentration to produce light. This is m/32000 for pyrogallol, 0.025 per cent for H_2O_2, 0.1 per cent for *dried* blood and 5 per cent for potato juice (i.e., 5 parts pure potato juice to 95 parts water).

Note also from tables 2 and 3 that very often the guaiac reaction can be obtained when, under similar conditions, no light production by pyrogallol takes place.

2. Substances taking the place of oxidase, pyrogallol and H_2O_2.

OXIDASE

Any plant extract containing strong oxidases such as occur in turnips, sweet potatoes, horse radish, etc., or any blood containing haemoglobin will give light with pyrogallol and H_2O_2. Apple juice, which oxidizes tannin, gives no light. Of many invertebrates, including insects, molluscs, annelids, crustaceans and echinoderms, only extracts of Chiton, a tubicolous annelid (*Laonome japonica*), and a polychaete (Nereis sp? —) have thus far been found to give light. The haemocyanin of crab or squid blood does not, nor do extracts of the luminous parts of luminous animals. Among these, bacteria, an annelid, (*Chaetopterus*), a squid (*Watasenia scintillans*), a crustacean (*Cypridina*), a protozoan (*Noctiluca*), a pennatulid (*Cavernularia*) and the fire-fly have been tried.

Among inorganic substances, m/20 $K_4Fe(CN)_6$ gives a bright light, m/10 $FeCl_3$ a fair light and m/40 $KMnO_4$ a faint light. Platinum black, bromine water, m/10 $MnCl_2$, m/10 $FeSO_4$, m/10 $K_2Cr_2O_7$, m/10 $K_3Fe(CN)_6$, m/10 $CuSO_4$, or Fehling's solution at 100° give no light. In greater concentration these substances may oxidize sufficiently to give light as does the mixture of pyrogallol, Na_2CO_3, H_2O_2 and formaldehyde described by M. Trautz (8).

PEROXIDE

Only H_2O_2 will give light with pyrogallol and oxidase. Na_2O_2, BaO_2 or ozonized turpentine do not, although they give a strong guaiac reaction, with the peroxidase of turnip juice.

We must add H_2O_2 to potato juice, which already contains a peroxide whose oxygen may be transferred to guaiac. It is no doubt in too small a concentration to give light with pyrogallol.

OXIDIZABLE SUBSTANCE

Many of the amino- and hydroxy-phenols are rapidly oxidized by potato juice + H_2O_2 but pyrogallol was the only one which gave light. The following were tried and all gave colored oxidation products: phenol, tricresol, pyrocatechin, resorcin, hydrochinon, orcinol, *a*-napthol, ortol, anilin, *o* and *p* toluidin, *a*-napthylamin, guaiacol, paraphenylen-diamine, amidol and pyramidon. Phloroglucin, *β*-napthol and *β*-naphtylamin were not oxidized.

Among the glucosides, vanillin, amygdalin, phloridzin, salicin, saponin and aesculin gave no light with potato juice either at 20° or 60°C.

3. Effect of temperature on the reaction.

A very faint light is produced when pyrogallol + H_2O_2 at 0° is mixed with potato juice at 0°C. The light is much brighter at 5° and quite bright at 10°C. Pyrogallol + H_2O_2 mixed with blood (1 per cent) gives no light at 0°, but a very faint light at 2°C.

High temperatures destroy the pyrogallol oxidase of potato juice. This occurs between 80° and 85° as indicated in table 4. The potato juice was rapidly heated to the temperatures 50°, 55°, 60°, etc., then cooled rapidly in running water. The destruction of catalase, the formation of a precipitate in the potato juice, and the effect on its power to blue guaiac alone, guaiac + H_2O_2 and guaiac + ozonized turpentine are also recorded. Similar results were obtained with tur-

TABLE 4

POTATO JUICE HEATED TO	LIGHT PRODUCTION WITH PYROGALLOL + H_2O_2	BLUEING OF GUAIAC	BLUEING OF GUAIAC + H_2O_2	BLUEING OF GUAIAC + OZONIZED TURPENTINE	EFFECT ON CATALASE. LIBERATION OF O FROM H_2O_2	FORMATION OF PRECIPITATE IN POTATO JUICE
deg.						
50	Bright	Blue	Blue	Blue	+++	None
55	Bright	Blue	Blue	Blue	+	None
60	Bright	Blue	Blue	Blue	None	None
70	Faint	Slight blueing	Fair blueing	Blue	None	Precipitate
80	Very, very faint	Very slight blueing	Slight blueing	Blue	None	Precipitate
85	None	None	None	Blue	None	Precipitate
90	None	None	None	Blue	None	Precipitate
100	None	None	None	Slight	None	Precipitate
100(10 min.)	None	None	None	None	None	Precipitate

nip juice. Blood does not lose its power to oxidize pyrogallol + H_2O_2 with light production or guaiac + H_2O_2 even after ten minutes boiling.

Note from table 4 that the pyrogallol and guaiac oxidases in presence of H_2O_2 are both destroyed between 80° and 85° but that with ozonized turpentine the juice heated to 100° still blues guaiac faintly. The natural peroxide (oxygenase) of potato juice which disappears on standing is destroyed more readily than the peroxidase. Catalase is destroyed between 55° and 60° before a precipitate has appeared, whereas peroxidase is still active after a precipitate has appeared.

4. Effect of KCN, NaOH, and HCl.

In testing the effect of these three substances equal parts of (1 cc. m/160 pyrogallol + 1 cc. 3 per cent H_2O_2) and (potato juice + KCN or NaOH or HCl) were mixed. The table (5) gives also the effect of

TABLE 5

SUBSTANCE	CONCENTRATION IN POTATO JUICE	LIGHT PRODUCTION WITH PYROGALLOL + H_2O_2	LIBERATION OF O FROM H_2O_2	BLUEING OF GUAIAC + H_2O_2	COLOR OF INDICATOR	INDICATOR
KCN	m/20	None	+	None	Yellow	Neutral Red
	m/40	None	+	Very faint blue	Orange	
	m/80	None	++	Fair blue	Orange	
	m/160	None	++	Fair blue	Red	
	m/320	None	++	Fair blue	Red	
	m/640	None	++	Fair blue	Red	
	m/1280	None	+++	Blue	Red	
	m/2560	Very faint	+++	Blue	Red	
	m/5120	Faint	+++	Blue	Red	
	m/10240	Fair	+++	Blue	Red	
	m/20480	Bright	+++	Blue	Red	
NaOH	m/20	None	+	None	Yellow	
	m/40	None	+++	Faint blue	Yellow	
	m/80	Faint	+++	Blue	Yellow orange	
	m/160	Bright	+++	Blue	Red orange	
	m/320	Bright	+++	Blue	Red	
HCl	m/20	None	None	None	Red orange	Dimethylaminoazobenzol
	m/40	None	None	Faint blue	Yellow	
	m/80	None	None	Faint blue	Yellow	
	m/160	Fair	+	Blue	Yellow	
	m/320	Bright	+++	Blue	Yellow	
	None	Bright	+++	Blue	Yellow	

catalase on the blueing of guaiac + H_2O_2, and the color of indicators added to determine the amount of acid or alkali uncombined with protein. Neutral red is red in neutral or acid solution, yellow in alkalies. "Di-methyl" is yellow in neutral or alkaline solution, red in free acid solution.

Note from table 5 that KCN affects the pyrogallol peroxidase in very weak concentration (m/2000) but is not so effective in destroying catalase or preventing the blueing of guaiac.

NaOH and HCl are not so toxic. As we might expect the HCl is the more toxic of the two.

5. Effect of fat solvent anaesthetics.

Saturation of the potato juice or blood with ether or chloroform does not affect its power to produce light with pyrogallol + H_2O_2. A sample of horse radish root extract preserved 45 days under ether gave a good light when mixed with pyrogallol + H_2O_2.

6. Is the oxidase a catalyser?

That oxidases in general are not catalysers but are used up in the reaction has been proven by the work of Kastle (9), who found that the amount of phenolphthalein oxidized is proportional to the quantity of blood present, and of Bach and Chodat (10), who found that the amount of pyrogallol oxidized to purpurogallin is proportional to the amount of peroxidase and also to the amount of H_2O_2 present but independent of the quantity of pyrogallol, providing the pyrogallol is present in excess of the quantity capable of being oxidized by peroxidase and H_2O_2. Melanin formation by tyrosinase is also proportional to the amount of tyrosinase present (11).

The peroxidase, therefore, transfers the O of H_2O_2 to the pyrogallol and the H_2O_2 pyrogallol and peroxidase are all changed, i.e., used up in the reaction. If we represent the peroxidase by P and the pyrogallol by B the reaction must take place as follows:

$$\begin{aligned} P \quad &+ \quad 2H_2O_2 = PO_2 \quad + 2H_2O \\ PO_2 &+ B \quad\quad = PO \quad + BO, \end{aligned} \tag{1}$$

and not as:

$$\begin{aligned} P \quad &+ 2H_2O = PO_2 + 2H_2O \\ PO_2 &+ 2B \quad = P \quad + 2BO \end{aligned} \tag{2}$$

Note that in the first equations the peroxidase (P) is used up and in the second equations it acts as a true catalyser and is regenerated again.

That the peroxidase of turnip juice is used up so far as its power to

cause light production in pyrogallol is concerned is indicated in the following experiment:

If 1 cc. m/10 pyrogallol + 4 cc. H_2O_2 (3 per cent) is mixed with 5 cc. turnip juice heated to 80° (to destroy the catalase and weaken the peroxidase), a very faint light is produced. A small amount of peroxidase is, therefore, present. The mixture is allowed to stand for 24 hours at 22°C. and then fresh turnip juice added. A good light results, showing that the pyrogallol has not been changed by a small amount of peroxidase when a long time has been allowed for the reaction to proceed. The pyrogallol was turned a light brown by the small amount of peroxidase and this color had not deepened in 24 hours, but did deepen immediately when the additional quantity of peroxidase was added. A small quantity of "enzyme" cannot, therefore, transform an indefinite amount of pyrogallol. A similar result was obtained with a weak haemoglobin solution, 0.1 per cent dried blood extract.

That a large quantity of peroxidase can be used up by addition of successive amounts of pyrogallol (i.e., by "titrating" the peroxidase) can be shown as follows:

To 10 cc. potato juice heated to 60° (to destroy catalase and consequent foaming due to liberation of O from H_2O_2) is added 2 cc. of a mixture of equal parts m/10 pyrogallol and 3 per cent H_2O_2. Light results. When this has disappeared (about 2–3 minutes) 2 more cubic centimeters of the pyrogallol-H_2O_2 mixture is added and a very faint light results. On adding a further 2 cc. no light appears. There is, however, in the final mixture plenty of pyrogallol + H_2O_2 to produce light as may be shown by adding fresh potato juice when abundant light appears. The peroxidase has been completely used up. A similar experiment with blood extract gave a similar result.

SUMMARY

1. Pyrogallol gives off a yellowish white light about equal in intensity to a suspension of luminous bacteria when oxidized by blood or plant juices (oxidases) in presence of H_2O_2.

2. The light is visible in very weak concentrations of pyrogallol, viz., m/32000 or 1 part to 254,000 parts solution. H_2O_2 must be present in at least 0.025 per cent, blood in 0.1 per cent (dried defibrinated blood) and potato juice in 5 per cent (fresh pure juice) to give a just visible light.

3. Of many easily oxidizable hydroxy- and amino-phenols only pyrogallol gives light. $K_4Fe(CN)_6$, $KMnO_4$ or $FeCl_3$ may take the place of the oxidase. Na_2O_2, BaO_2 or ozonized turpentine will not take the place of the H_2O_2. Animal extracts except those of some marine annelids and Chiton do not give light.

4. Light is produced at 0° and a bright light at 10°C. The oxidase of plant juices is destroyed between 80° and 85°.

5. KCN inhibits the light production in very weak concentration, m/1280 to m/2560. NaOH inhibits in n/40 and HCl in n/80 concentration.

6. Ether and chloroform have no effect on the light production.

7. The oxidase is not a true catalyser but is used up in transferring oxygen from H_2O_2 to the pyrogallol.

8. The reaction is compared with the production of light by luminous animals which it mimics very closely.

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THE CORPUSCLES, HEMOGLOBIN CONTENT AND SPECIFIC GRAVITY OF THE BLOOD DURING HIBERNATION IN THE WOODCHUCK (MARMOTA MONAX)

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INTRODUCTION

The fact that a great many physiological and pathological conditions are attended by a variation in the number of corpuscles and in other constituents of the blood, has naturally led to an investigation of the effect of hibernation on the composition of the blood. It is well known that during this period of lethargy, lasting three or four months and even longer, there may be almost continuous sleep and there may have been no ingestion of food or water whatsoever. In consequence there is a loss in the body weight amounting to as much as one fourth. The body temperature may fall from 38°C. to only a few degrees above 0°C. and practically all the vital processes are reduced far beyond that met with in many other conditions that are known to have a marked effect on the blood.

Vierordt (1) was not only a pioneer in the accurate counting of blood corpuscles, but also the first to describe a change in their number during hibernation. In the marmot he found 5,800,000 red corpuscles per c.mm. at the beginning of winter-sleep, while at the end of this period he found only 2,300,000. According to Valentin, (2) he also found that the corpuscles diminished in size as well as in number. Quincke (3) similarly found at the end of hibernation (May 17) in the marmot only 3,500,000 red cells, or 70% of the number found during the summer. Hemoglobin determinations showed only 8.8 per cent or 64 per cent of the normal amount of this pigment. Quincke took the blood from the pulsating heart. He found the erythrocytes to be of the usual form but of various sizes, and some of them contained yellow droplets.

Quincke thought that he saw in the spleen, red bone marrow and liver, during hibernation, abundant evidence of the disintegration of red corpuscles. In the capillaries of the liver, for example he found leucocytes containing iron-holding granules, especially in old marmots. These granules were blackened with sulphide of ammonium. He concluded that the iron from the broken down red cells must accumulate in these organs and later serve as a source of iron in the regeneration of new corpuscles. These pigment granules containing iron were later observed by Carlier (4) to be present in the bile capillaries in the hibernating hedgehog. He also found in the liver and in the large veins coming to the liver, large rounded or oval mononucleated cells, the cytoplasm of which often contained iron-holding pigment. He believed that these large cells were macrophages which had wandered into the liver after having absorbed pigment resulting from the destruction of erythrocytes. Unlike Quincke, however, he does not think that this pigment is stored in the liver for future use, but that it simply accumulates there owing to the sluggish state of the excretory function of that organ during hibernation. Later the pigment is most likely excreted as usual, since he found that the liver did not appear to be a blood-producing organ after the animal wakes up from winter-sleep.

The red corpuscles were also counted by Dubois (5) in a number of marmots at various times from November to May. His table of results shows that with the progress of hibernation there is a slight increase in the number of erythrocytes, both in arterial and in venous blood (the blood being from the larger vessels), until towards the end of this period, at which time there is a decrease from over 5,000,000 to less than 3,000,000 per c.mm. while the animal is still torpid, and to about 2,000,000 when the animal wakes up. In two cases the exact figures may be given with profit.

ANIMAL	DATE OF ENUMERATION	CONDITION OF ANIMAL	RECTAL TEMPERATURE—C	ARTERIAL BLOOD	VENOUS BLOOD	PORTAL BLOOD
I	February 12...	Torpid	5.6	5,320,000	5,765,000	5,675,000
	February 15...	Awake	34.2	4,730,000	4,905,000	5,885,000
II	May 11.....	Torpid	15.0	2,930,000	2,540,000	
	May 11.....	Awake	35.0	2,070,000	2,530,000	

The extremely low figures for the animal examined on May 11 were checked up by hemoglobin determinations, which showed only 8.5

per cent hemoglobin in the arterial blood and 7.50 per cent to 7.75 per cent in the venous blood while the animal was asleep with a rectal temperature of 15°C. When this same animal woke up on the same day and the body temperature had increased to 35°C. there was still less hemoglobin, especially in the arterial blood. The figures given are 7.25 per cent to 7.50 per cent for both the venous and the arterial blood. The hemoglobin determination thus checks well with the "blood count," since at the beginning of hibernation there was about double this amount of hemoglobin.

It appears from these figures, therefore, that there is a distinct decrease in the number of red corpuscles when the animal wakes up both during mid-hibernation and at the end of this period.

The gradual increase in the number of erythrocytes at the first progress of hibernation which Dubois noted, he attributes to an increase in the specific gravity of the blood due to loss of water, for he found the density of whole arterial blood of the hibernating marmot to be 1.071 and defibrinated arterial blood, 1.0654. The corresponding figures for the awake marmot are 1.046 and 1.0525. The great decrease in the number of red corpuscles at the end of winter-sleep he takes to indicate that there is an actual destruction of these cells.

Polimanti (6) gives the following table as the results of the enumeration of the red corpuscles of four marmots by means of the Thoma-Zeiss apparatus:

ANIMAL	RECTAL TEMPERATURE—C	CONDITION OF ANIMAL AT THE TIME OF ENUMERATION	BLOOD FROM CAROTID ARTERY	BLOOD FROM JUGULAR VEIN	BLOOD FROM PORTAL VEIN
I	10.5	Dormant 15 days	5 250,000	5,450,000	5,620,000
II	7.5	Dormant 8 days	5,350,000	5,565,000	5,700,000
III	9.0	Dormant 20 days	5,400,000	5,610,000	5,780,000
IV	10.8	Dormant 30 days	5,500,000	5,760,000	5,850,000
I	36.0	Awake, July	4,540,000	4,900,000	5,150,000
II	36.5	Awake, at the end of hibernation	4,500,000	4,950,000	5,200,000
III	36.4	Awake, October	4,650,000	5,000,000	5,150,000
IV	36.7	Awake, September	4,700,000	5,100,000	5,200,000

His figures on the hemoglobin content of the blood of four marmots during winter-sleep and again when awake show an average per cent of 18.62 in carotid blood, 11.06 in jugular blood and 25.82 in portal blood during hibernation, and 15.905, 15.46 and 21.22 respectively

when awake and active. The blood from the jugular vein is the only case where during hibernation there is less hemoglobin than during the waking state. Besides indicating a greater percentage of hemoglobin in the arterial and the portal blood during hibernation, and thus in general verifying the corpuscle count, these figures show an increasing quantity of hemoglobin as you go from the jugular vein to the carotid artery and finally to the portal vein. This, he thinks, indicates that there is an unequal distribution of the red corpuscles in the various vascular zones. His table given above shows that in this particular the carotid and jugular blood ought to be interchanged in order to agree with the hemoglobin determinations. Incidentally it might be noted that the erythrocytes are most numerous in the portal blood. Upon the theory that the increase in the number of red cells following exercise and other factors that increase blood pressure is due to bringing into circulation more of the corpuscles that tend to settle down in the splanchnic area during a slower circulation (7), one might expect even a comparatively greater number of erythrocytes in the portal blood during such a sluggish circulation as is seen in hibernation.

Polimanti's figures are in general agreement with those of the previous authors, except that the decrease at the end of winter-sleep is not nearly so great as reported by them. He, like Dubois, considers the slight increase during the progress of hibernation as being only apparent and as explainable by the increase in the specific gravity of the blood. He found in the case of three marmots that the density of the blood on November 15, 1895, during the normal waking state was 1.045, 1.050 and 1.037. On March 10, 1896, the corresponding figures were: 1.080, 1.075 and 1.065. At this latter date the animals were hibernating. That the blood loses water during hibernation is indicated by the work of Aeby (8) who analysed the various tissues and organs of the marmot for the water and the ash contents during the early part of hibernation and again during the latter part of this period. He found the blood in the early part of winter-sleep to contain 83 per cent water, 17 per cent dried solids, 0.87 per cent ash in the fresh blood and 5.11 per cent ash in the dried solids. In the latter part of the dormant period the corresponding figures are 78.63 per cent water, 21.37 per cent dried solids, 0.91 per cent ash in the fresh blood and 4.26 per cent ash in the dried solids. Dubois obtained venous and arterial blood from the femoral vessels and dried in at a temperature of 100°C. over sulphuric acid and found 84.51 per cent water in the arterial blood and 83.70 per cent water in the venous blood of an

active marmot with a rectal temperature of 35°C. In a dormant marmot with a rectal temperature of 11°C., the corresponding figures obtained were 79.76 per cent water in the arterial and 80.23 per cent in the venous blood. This is an average loss of about 4.11 per cent. Polimanti similarly observed a decrease in the water content of the blood of the hibernating marmot amounting to about 4.6 per cent.

In regard to the white corpuscles, Quincke found that they were present in fair quantities during hibernation and were not practically absent as was stated by Valentin. According to Quincke, Valentin explained this reduction in the white cells as being due to the absence of lymph, which he thought conveyed the leucocytes into the blood stream. A table of the actual number of white corpuscles found by Dubois (wrongly attributed to Quincke by Polimanti, p. 186) in the marmot shows that in the carotid, jugular and portal blood of a hibernating animal with a rectal temperature of 8.6°C. there were respectively 4,000, 7000 and 9000 leucocytes; while in an active animal with a rectal temperature of 34.2°C. the corresponding numbers were 7000, 12,000 and 9000. It appears from this that the arterial blood is especially poor in white corpuscles during winter-sleep. The increase in the number of white cells in the blood vascular system generally and especially in the veins in the active state is explained by Dubois as being due to the greatly increased circulation of peritoneal lymph at the time of waking up. The fact that there is no such increase in the portal blood at this time he thinks is probably due to the fact that the large lymphatic trunks about the portal vein do not empty into the portal vein, but course directly into the liver.

Carlier (p. 97) found that in the hedgehog during hibernation there is a great increase in the number of wandering cells in the connective tissue of the mucous and submucous coats of the stomach and in some other places. He thinks that these cells are white corpuscles that have migrated from the circulating blood. He cites in support of this idea the fact that normally during the active state the blood of this animal contains from 18,000 to 20,000 leucocytes per c.mm., but the number falls suddenly at the very commencement of winter-sleep to from 1000 to 3000. At the same time he noted that the red corpuscles show, if anything, rather an increase in number. Polimanti similarly showed that in the marmot there is a marked decrease in the number of circulating white corpuscles, which he also explains as being due to diapedesis which always occurs during hibernation and which is also responsible for the large amount of lymph found in the

peritoneal cavity in winter-sleep. His figures for the same animals and under the same conditions as those mentioned in connection with his red count, are strictly in agreement with those given by Dubois and show an average of 3850, 6898 and 8663 per c.mm. of blood from the carotid artery, jugular vein and portal vein respectively, during hibernation; while the corresponding figures for various periods of the active stage are: 6535, 11,148 and 9020.

All the data that have been found so far on this subject seem to warrant the conclusion that during hibernation in warm blooded animals there is a great reduction in the number of circulating leucocytes. In the European marmot, which has been investigated most thoroughly, this reduction amounts to about one half.

Very little work has been done on differential counting of the white corpuscles during hibernation. Argaud and Billard (9) induced dormice to hibernate in the summer by depriving them of food. After ten days of such sleep, they found only a few white cells in the blood and these were of the mononuclear type.

It is interesting in this connection to note that, while there is a great deal of disagreement in the results of blood examination during long inanition in man and other animals, many authors report striking alterations in the number and formula of the leucocytes, indicating in general that there is a decrease; a slight absolute loss of both red corpuscles and hemoglobin, although these elements seem to be very resistant; a tendency towards a concentration of the blood and hence an increase in the specific gravity due to the loss of water, with a slight relative increase in hemoglobin and erythrocytes. Upon refeeding there is a relative decrease in the hemoglobin, red corpuscles and specific gravity due to the acquisition by the blood of water faster than other elements. Yet a month of fasting (water being allowed) by man may show surprisingly little or no change in these respects (10).

PRESENT INVESTIGATION

The work reported here is based upon observations carried out during the last three years on the woodchuck (Marmota monax), a rodent which hibernates profoundly three or four months each year without having anything to eat or drink during this time. These animals were captured uninjured in this vicinity (Ithaca, N. Y.), and were kept in the artificial burrows designed by Dr. Simpson of this laboratory and described in detail elsewhere (11). Many of the animals were used

a few days after being captured. While in captivity they were supplied with abundant food and water. Those investigated while dormant were obtained usually in September and fed till hibernation set in. The conditions of the artificial burrows are such that there is no reason to believe that the animals kept in them will not be representative of those in the natural burrows. Some of the animals were kept till spring and allowed to wake up. A number of these animals were investigated before they had recommenced to feed and others were fed for some time and then examined. A number of animals were also obtained fresh from the field in the early spring after feeding had commenced. A much larger number of animals would have been desirable especially during some of the stages, but due to the escape of 32 animals of one series through a hole accidentally or maliciously made in the metal wall of the inclosure at a late date in autumn when no more could be obtained, and the death of about 15 of another series during the fall and winter, the number of animals was unintentionally decreased.

METHOD.

The active animal was rapidly anaesthetized with ether by being put into a small box in which ether had been placed some time previously. The onset of anaesthesia was rapid and not attended with marked struggling. Since the duration of the ether anaesthesia before the blood determinations were made was, as a rule, less than twenty minutes, there ought to be but little error in the relative results from these disturbing factors. The dormant animal was either not given any anaesthesia at all, or was given chloretone one hour before the operation and was kept undisturbed in a basket of straw or, if practicable, in its habitual burrow until the operation was commenced. It was found necessary to give, even to the active animal, twice the dose prescribed for the rabbit. In order not to introduce too much liquid into the rectum and thus dilute the blood appreciably, 2 cc. of 50 per cent alcohol containing 0.3 gram of chloretone was given per rectum for each kilogram of body weight. This did not disturb the animal noticeably and the blood determination from such animals compared well with those not given any anaesthetic. Chloretone was given in order not to disturb the animal by the preliminary operation involved in exposing the vessels, since the object was to determine the condition of the blood in the dormant animal, and since after the blood determinations, other experiments were frequently planned which de-

manded an undisturbed animal. The use of a little cocaine as a local anaesthetic while isolating the vessels was also resorted to in some cases.

Semi-dormant animals were either given chloretone, aided by cocaine as a local anaesthetic, or ether. This point will be discussed in connection with the results.

Blood was obtained from the carotid artery (in a very few cases from the femoral artery) by means of a glass cannula and a short rubber tube. The cannula and rubber tube were washed out by allowing a little blood to escape and then from 1 to 2 cc. of blood was collected in a small paraffined vessel. The pipettes of the Thoma-Zeiss haemocytometer and the Gowers-Haldane haemoglobinometer were then filled in rapid succession and four cover glass smears were made. These smears were later stained with Wright's blood stain. The cannula and rubber tube were again flushed out with blood and a fresh supply amounting to nearly 6 cc. was collected in a small specific gravity bottle. The stopper was carefully inserted so as to inclose no air bubbles and the bottle wiped free from blood on the outside with a moist cloth. The weight of the blood was compared with an equal volume of distilled water at the same temperature. Thus a very accurate determination of the specific gravity was possible.

In case of the erythrocytes at least 400 and in case of the leucocytes at least 800 squares were counted, and at least two samples were taken from the pipette. The same counting chamber, cover glass and pipettes were used through the entire series, and furthermore the cover-glass was placed with the same side down and as nearly in the same position as could be judged by the outline of the square coverglass which had been scratched on the counting chamber. With these and the other usual precautions necessary to the obtaining of reliable results, together with the advantage of having an ample supply of fresh blood direct from a large artery and plenty of time to fill the pipette accurately, the figures obtained are believed to be free from serious error.

The Gowers-Haldane clinical method of hemoglobin determination was considered sufficiently accurate under the conditions, and hence the results are really in percentages of normal oxygen capacity (18.5 volumetric per cent) of man, which usually means about 14 per cent hemoglobin. Since no standard for the woodchuck exists, only the relative amount of hemoglobin is indicated.

RESULTS

The results are placed in the tables below.

Group I. Before hibernation. Animals active

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R. T.C.	R. B.W.	White	Red		
1913								
November 22....	2–SI	♀	37.2	2440	18,440	6,229,600	109	1.0591
December 13....	14–SI	♂	35.8	2650	12,132	5,864,000	100	1.0597
1915								
July 8.....	5–SIII	♀	36.1	2757	14,000	7,748,000	118	1.0639
July 9.....	6–SIII	♀	38.4	2464	12,300	5,542,000	100	1.0569
July 20.....	12–SIII	♂	36.7	2690	10,800	5,268,000	90	1.0555
July 29.....	17–SIII	♀	36.7	2760	3,900	5,464,000	94	1.0540
August 2.....	19–SIII	♀	36.7	2771	10,300	6,106,000	110	1.0627
August 5.....	20–SIII	♂	38.5	3190	11,700	6,263,000	110	1.0625
August 20.....	26–SIII	♀	38.3	3130	13,500	6,580,000	108	1.0641
August 24.....	28–SIII	♀	37.2	2284	15,500	6,075,000	100	1.0558
August 26.....	29–SIII	♀	36.9	2230	13,000	7,418,000	114	1.0633
November 6.....	37–SIII	♀	36.5	1725	18,450	7,298,600	114	1.0606
November 20....	39–SIII	♂	36.0	1730	9,950	5,150,000	90	1.0566
November 23....	40–SIII	♀	37.0	1622	6,550	5,942,000	98	1.0577
Average.....	.....	.....	.....	2460	12,180	6,210,586	103.9	1.05946

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group II. During hibernation. Animals dormant.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R. T.C.	R. B.W.	White	Red		
1914								
February 3.....	5–SI	♂	8	2225	6,300	5,357,000	99	1.0587
February 28.....	7–SI	♀	12	2475	7,600	5,641,000	95	1.0599
March 14.....	9–SI	♀	11	1375	5,432	5,976,700	100	1.0594
1915								
December 18....	42–SIII	♀	9	2100	4,360	5,832,000	100	1.0554
December 28....	45–SIII	♀	10	2046	10,050	7,949,000	115	1.0647
1916								
February 7.....	48–SIII	♂	6	813	800	6,678,000	100	1.0600
February 26.....	51–SIII	♀	7	1635	7,200	6,928,000	108	1.0581
March 4.....	52–SIII	♂	10	1203	5,050	6,768,000	102	1.0565
March 6.....	53–SIII	♂	12	1849	6,800	7,796,000	114	1.0628
Average.....	.....	.....	.....	1747	5,955	6,547,300	103.7	1.0595

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group III. During hibernation. Animals sluggish or semi-dormant

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R.T.C.	R.B.W.	White	Red		
1915								
December 21....	43–SIII	♂	19	2525	34,000	7,606,000	118	1.0654
1916								
February 8.....	49–SIII	♂	23	1615	22,250	7,524,000	114	1.0608
April 1.....	55–SIII	♂	24	1355	19,400	7,528,000	110	1.0625
Average.....				1832	25,217	7,552,667	114	1.0629

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group IV. After hibernation. Animals awake and active. Nothing to eat or drink.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R.T.C.	R.B.W.	White	Red		
1914								
March 18.....	11–SI	♀	36	1325	12,266	6,666,000	114	1.0600
March 25.....	13–SI	♂	34	2175	8,600	6,556,000	116	1.0628
March 28.....	14–SI	♂	35	1725	4,767	6,934,000	115	1.0648
1916								
February 25.....	50–SIII	♀	35	2936	7,750	7,363,000	116	1.0659
April 3.....	56–SIII	♂	34	2210	9,650	7,338,000	109	1.0612
Average.....				2074	8,607	6,971,400	114	1.06294

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group V. After hibernation. Animals awake and active. Fed.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R.T.C.	R.B.W.	White	Red		
1914								
April 5.....	15–SI	♂	36	1803	9,067	5,506,000	92	1.0539
1915								
May 18.....	14–SII	♀	37.7	1550	7,200	4,880,000	82	1.0489
1916								
April 22.....	59–SIII	♂	36	1775	5,400	5,176,000	90	1.0515
April 24.....	60–SIII	♀	37.5	1175	13,550	4,936,000	85	1.0493
April 28.....	61–SIII	♀	35	1511	11,700	7,416,000	115	1.0611
May 4.....	64–SIII	♀	37	1695	5,800	4,644,000	75	1.0480
May 10.....	66–SIII	♂	37	2420	10,800	5,328,000	100	1.0569
May 12.....	67–SIII	♀	38	1690	17,600	5,094,000	84	1.0491
May 13.....	68–SIII	♀	38.5	1810	11,450	5,188,000	85	1.0491
Average.....				1714	10,285	5,352,000	89.8	1.0520
Average-Excluding No. 61–SIII.....					10,108	5,094,000	86.6	1.0508

R. B. W., Reduced body weight. R. T., Rectal temperature.

An examination of the above results shows that in fourteen woodchucks, in the active state, during the summer and autumn, the number of red corpuscles per c.mm. varies from 5,150,000 to 7,748,000, with an average of 6,210,586. The relative amount of hemoglobin varies from 90 to 118, with an average of 103.9. The specific gravity ranges from 1.0540 to 1.0641, with an average of 1.0595. If the specific gravity of the blood of seven other animals, used for other experiments, be considered, the limits in a total of twenty-one animals are 1.0540 and 1.0658 with practically the same average, or 1.0596. The individual variations in the erythrocytes, hemoglobin and specific gravity usually run more or less parallel in this as well as in the other groups. The white corpuscles range from 3900 to 18,450, with an average of 12,180. Only in one case is there any exceptional departure from the usual variations characteristic of a leucocyte count.

From the figures in the second group of nine dormant animals which were examined at various intervals during hibernation (December to March) it is evident that in the case of the red cells there are about the same limits to the variations and an average of 6,547,300, or about 5 per cent more than during the active state before hibernation; while the average specific gravity and the average amount of hemoglobin remain practically the same as in the first group. By including three more animals, the specific gravity of whose blood was examined while dormant, the average is raised slightly or to 1.0599. The fact that the average body weight in this group is only 1747 grams as compared with 2460 grams in the first or active group does not mean that the dormant animals were much smaller and hence much younger. There is in the dormant group only one especially young animal (as judged from its weight of only 813 grams) and in this case the specific gravity and number of red corpuscles are above the average for the group, while the hemoglobin is less than the average by only slightly more than the probable error. It must be remembered that during hibernation the animal loses at least one fourth of its weight. In short, the great increase in the specific gravity and in the amount of hemoglobin of the blood during winter-sleep as stated by Dubois and Polimanti in regard to the European marmot, does not apparently take place in the woodchuck. Nor is there a very marked increase in the red corpuscles.

An entirely different picture is, however, presented by the disposition of the white corpuscles. The table shows that there is a variation from 800 to 10,050, with an average of 5955. With the exceptions of

the two cases which mark the extreme variations, the individual counts are surprisingly close to the average for the group. Thus during hibernation there is in the woodchuck, as in other animals investigated, an enormous decrease in the number of circulating leucocytes. In this case, as in the European marmot, the number falls to about one half the number found during the summer. This has been explained by Dubois, Carlier and Polimanti as being due to diapedesis. In this connection might be mentioned a theory advanced by Hansemann (12) who, upon examination of the various organs of animals killed while hibernating, found no evidence of cell division. He concluded that cell division normally occurs as a result of the wearing out of the tissues. When activity is greatly reduced, as in winter-sleep, the stimulus is gone and there is no cell division. In accordance with this theory, there might be a decrease in the production of leucocytes, which would also contribute to the reduced number of circulating white corpuscles.

A few semi-dormant animals were examined during the hibernating period and found to present such a marked contrast to the more torpid ones that they have been placed in a separate group. Since there are only three individuals not much can be based upon them, but it did not seem justifiable to include them with the more dormant ones. These animals had a rectal temperature from 19° C. to 24°C. No. 43 was beginning to hibernate. No. 49 was either going back to sleep or coming out of it. No. 55 was waking up from winter-sleep. The red corpuscles are increased 1,000,000, or more, and the hemoglobin and specific gravity likewise show the maximum average figures of 114 and 1.06294 respectively. Still more marked is the large number of white corpuscles which average 25,217, or more than double that of any corresponding figure. Since these animals were moving about, it was necessary to employ some anaesthetic. Ether was used on No. 43. While anaesthesia set in rapidly and had only been continued ten minutes before the blood sample was taken, it was considered probable that the high figures were in some way, at least partly, due to the ether. The next animal, No. 49, was given chloretone with as little disturbance as possible; but all the figures were still high. On the third animal, No. 55, ether was tried again, with somewhat similar results. It appears that in this transitional stage, there is an enormous number of leucocytes circulating in the blood. It might be that there is some irritation or struggle within the organism, such as resistance against the cold, during the transition which causes leucocytosis and

a dislodging of corpuscles, both red and white, which had settled out of circulation. The high specific gravity would suggest that enough activity is going on to cause a loss of water by the blood, somewhat the same as apparently occurs after the animal becomes fully awake and active and has no access to food or water.

In the fourth group there are included those animals which have become fully awake after hibernation and have not had anything to eat or drink. They had been awake from two days to two weeks. Here again the group is rather limited, involving only five animals, but the results are strikingly uniform and opposed to what one might expect from the literature. The red corpuscles average nearly 7,000,000, which is fully 500,000 more than was found during torpor, and a little more than 500,000, or 8 per cent, less than was found in the semi-dormant animals. The specific gravity and the amount of hemoglobin show the same average as was found in the third group, and suggest a concentration of the blood by the loss of water due to increased activity and a much greater difference between the body temperature and the temperature of the surroundings. Thus it has been fully demonstrated by the extensive observations of Jones (13) that in the human subject exercise causes a high specific gravity of the blood. The woodchuck shows no evidence of the great decrease to one-half the normal number of erythrocytes when the animal wakes up from lethargy, such as is described by Vierordt, Quincke and Dubois in connection with the European marmot. This decrease, then, is not always characteristic of hibernating animals when they first wake up, for exactly the opposite condition prevails in the woodchuck.

The number of white corpuscles is very uniform and they are somewhat more numerous than during dormancy, but not as numerous as during the active period in summer and autumn, while they are only one third as numerous as in the third or sluggish group.

Finally, there is the series of nine animals which have had access to food and water for one to at least six weeks either in confinement or in their natural habitat. The red corpuscles show a sudden drop of over one and a half million, or over 20 per cent, as compared with the animals that have not been fed. The hemoglobin similarly shows a decrease of over 20 per cent. The specific gravity likewise decreases to 1.0520, indicating that the blood has been diluted from having taken up water faster than the other constituents. It is possible that the sudden decrease in the red corpuscles noted by several authors in the

European marmot may be due partly, at least, to the same cause. The results obtained from the woodchuck are very uniform with a single exception, No. 61. This animal was caught by some boys one night in a trap. It was taken to the public school the next day and played with till evening when it was brought to the laboratory. It was placed in a box containing food and water. A dark secluded box communicates with the feed box and into this the animal soon found its way. It was operated upon the next day and from the appearance of the intestinal contents it was evident that it had not eaten any of the food in the box. It was impossible to judge as to the drinking of water, since there were other woodchucks in the same box. There are only 64 grams of feces in the alimentary tract and this was unusually solid. As a rule, there is about 200 grams in such animals. It is, therefore, very probable that the struggling and excitement of the previous day had concentrated the blood by causing an excessive loss of water. However, it is included in the figures already mentioned. The average for Group V, excluding this questionable one, is also given. Excluding animal No. 61, the red corpuscles vary within the comparatively narrow limits of 4,644,000 and 5,506,000, with an average of 5,094,000, or about 18 per cent lower than is found later in the summer. Undoubtedly with the onset of warm weather these low figures will approach those found in Group I. The hemoglobin ranges from 75 to 100, with an average of 86.6, while the specific gravity has fallen to an average of only 1.0508, and within the limits of 1.0480 and 1.0569.

The white corpuscles in this group have increased from 8607 (in Group IV) to 10,285 or until they are nearly as numerous as later in the year.

Undoubtedly most of the variations (except those in the leucocytes) are dependent to a large extent upon the concentration of the blood and redistribution of the corpuscles. Sufficient data are not yet available on the changes in blood volume to determine what absolute changes are incident to hibernation.

The size of the red corpuscles does not vary appreciably as will be seen from the following tabulated averages of the measurement of the diameter of 100 corpuscles from each of 30 animals. Each group of 10 animals consists of 5 males and 5 females.

BEFORE HIBERNATION August–December	DURING HIBERNATION December–March	AFTER HIBERNATION March–May
Microns	*Microns*	*Microns*
7.36	7.37	7.39
7.34	7.33	7.37
7.28	7.27	7.36
7.27	7.24	7.29
7.25	7.23	7.27
7.24	7.22	7.23
7.23	7.22	7.19
7.18	7.18	7.10
7.14	7.15	7.06
7.01	7.03	7.01
Average....7.230	7.224	7.227

The above measurements were made on the stained film preparations by means of a filar micrometer ocular. Only perfectly shaped corpuscles encountered in the thinnest parts of the films were utilized. Care was taken to select portions of the film in which the corpuscles were well spread and which had dried quickly as judged by the preservation of the blood plates. Since four films from each animal were available, the perfect erythrocytes found within certain limits in the field were taken, commencing at one side of the area and going towards the other, in a regular order until twenty-five corpuscles had been measured. This procedure was repeated on the other three duplicate preparations. The average of all the one hundred measurements was taken as the size of the corpuscles in that particular animal.

In each animal the dried red corpuscles usually varied from 5.8μ to 9.2μ in diameter. Only a very few cells, however, were at or near these extremes.

The results of a differential count of the white corpuscles, utilizing again the stained films, are given in the following table. At least 500 corpuscles were examined in each case.

The percentage of lymphocytes in the group of eight dormant animals varied greatly (from 7.8 per cent to 42 per cent), as did also that of the neutrophiles (from 84.7 per cent to 36.3 per cent). The other groups showed less individual variations in these particulars. In the group of five active animals examined after hibernation but before they had recommenced to feed, there is an unusually large individual variation in the relative number of eosinophiles (from 0.2 per cent to 19.4 per cent), and a rather high average (11.4 per cent).

ANIMAL	LYMPHOCYTES (LARGE AND SMALL)	MONONUCLEAR AND TRANSITIONAL LEUCOCYTES	POLYMORPHO-NUCLEAR LEUCOCYTES		
			Neutrophilic	Eosinophilic	Basophilic
Before hibernation (August–November), animals active					
19–SIII	34.2	6.7	57.6	1.2	0.3
20–SIII	33.4	6.6	56.6	3.4	0.0
26–SIII	24.8	5.8	64.7	4.3	0.4
28–SIII	21.6	3.6	71.4	3.2	0.2
29–SIII	39.7	9.0	42.2	8.7	0.4
37–SIII	25.9	5.2	62.4	6.5	0.0
39–SIII	33.1	7.9	56.4	2.6	0.0
40–SIII	17.9	3.8	71.0	7.1	0.2
Average.....	28.8	6.1	60.3	4.6	0.2
During hibernation (December–March), animals dormant					
5–SI	25.6	4.4	66.1	3.5	0.4
7–SI	34.1	4.1	54.1	7.0	0.7
9–SI	42.0	8.9	36.3	12.3	0.5
45–SIII	11.5	6.0	76.7	5.6	0.2
48–SIII	13.4	8.1	76.6	1.9	0.0
51–SIII	7.8	3.5	84.7	4.0	0.0
52–SIII	10.0	5.8	79.4	4.8	0.0
53–SIII	19.7	6.2	67.5	6.6	0.0
Average.....	20.5	5.9	67.7	5.7	0.2
During hibernation, animals sluggish or semi-dormant					
43–SIII	17.2	8.5	68.9	5.4	0.0
49–SIII	26.8	9.0	58.1	6.1	0.0
55–SIII	28.3	4.7	56.5	9.0	1.5
Average.....	24.1	7.4	61.2	6.8	0.5
After hibernation (February–April) animals active; nothing to eat or drink					
11–SI	37.7	13.9	28.5	19.4	0.5
13–SI	21.9	7.4	54.1	16.4	0.2
14–SI	30.3	5.9	45.8	17.3	0.7
50–SIII	42.2	4.4	52.8	0.2	0.4
56–SIII	43.6	8.8	43.3	3.5	0.8
Average.....	35.1	8.1	44.9	11.4	0.5

ANIMAL	LYMPHOCYTES (LARGE AND SMALL)	MONONUCLEAR AND TRANSITIONAL LEUCOCYTES	POLYMORPHO-NUCLEAR LEUCOCYTES		
			Neutrophilic	Eosinophilic	Basophilic
After hibernation (April–May), animals active, fed					
15–SI	39.3	6.3	43.9	10.2	0.3
59–SIII	27.1	5.4	63.3	3.6	0.6
60–SIII	27.6	5.8	50.2	15.9	0.5
61–SIII	19.4	4.0	71.5	5.1	0.0
64–SIII	46.6	6.3	40.8	6.3	0.0
66–SIII	29.8	9.4	51.3	7.8	1.7
67–SIII	21.9	10.3	57.3	7.6	2.9
68–SIII	42.7	6.3	39.2	10.9	0.9
Average.....	31.8	6.7	52.2	8.4	0.9

It is evident that in hibernation in the woodchuck there is no such inversion of the leucocyte formula as was reported by Argaud and Billard to occur in the dormouse when induced to hibernate. On the contrary, there appear to be relatively fewer lymphocytes circulating during dormancy and semi-dormancy than during the active state, either before or after hibernation. This decrease is undoubtedly due to a much slower circulation of lymph in this torpid state.

A comparison of the group of animals that had been fed with those that had not been fed but were awake after hibernation, bears out the general statement that in herbivorous animals there is no digestion lymphocytosis.

Blood plates (thrombocytes) are very abundant at all seasons of the year.

SUMMARY

1. In the summer and autumn there are in the arterial blood of the woodchuck on an average, in round numbers, about 6,200,000 red corpuscles, 12,000 white corpuscles, 104 per cent hemoglobin (Gowers-Haldane standard), and a specific gravity of 1.0595.

2. There is very little change in the number of red corpuscles, amount of hemoglobin and specific gravity of the arterial blood in the dormant woodchuck during hibernation. The figures for the erythrocytes do show an increase of about 5 per cent.

3. The number of circulating white corpuscles decrease to about one half in the torpid animal during hibernation.

4. The cellular elements (erythrocytes and leucocytes), the amount

of hemoglobin and the specific gravity increase greatly in animals which during the hibernating period are only semi-dormant and have an intermediate body temperature. The white corpuscles are four times as numerous as in the dormant animal and twice as abundant as in the active animal before hibernation.

5. After the animal wakes and becomes active, but before it has access to food, there is the same high specific gravity and large amount of hemoglobin as is found in the semi-dormant animals. The red corpuscles, however, are about 8 per cent lower, but this is still about 6 per cent higher than during the torpid state. The leucocytes are sub-normal, though somewhat more numerous than during profound lethargy.

6. There is a decided decrease, amounting to about 20 per cent, in the number of red corpuscles and the amount of hemoglobin after the awakened animal has commenced to eat and drink. The specific gravity greatly decreases also, suggesting that these variations are largely due to the relative concentration of the blood. The white corpuscles, on the other hand, increase to nearly normal.

7. The red corpuscles do not change in size during hibernation.

8. The leucocyte formula is not inverted with respect to the relative number of mononuclear and polymorphonuclear forms. There is, if anything, a decrease in the mononuclear types during winter-sleep due to a relatively greater decrease in the number of circulating lymphocytes.

It is with great pleasure that I acknowledge the credit due Dr. Sutherland Simpson for the opportunity of carrying on this research and for the suggestions and encouragement received at all times. The valuable help rendered by Gertrude B. Rasmussen is also worthy of mention.

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THE DIASTASE IN THE SALIVA OF THE OX

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INTRODUCTION

The importance of the digestive action of the saliva of man on starch was long underestimated, but it is now known to be of considerable importance. Its importance is due to the rapidity of the action and to its continued activity after food reached the stomach. It was formerly thought that the action was checked at once on entrance of food into the stomach. We now know that this is not the case, but that the food collects in the fundus of the stomach in a large mass, in the interior of which the reaction is very faintly acid. The optimum conditions for ptyalin action prevail and for a half hour or longer, depending on the size of the meal, the ptyalin may continue to digest the starches. Some workers are also of the belief that the presence of saliva, or its digestive products, may also act as a stimulus to the secretion of gastric juice.

The question of the presence of a starch splitting enzyme in the saliva of the ox is a disputed one. Ellenberger (1) states that Speichel-diastase (ptyalin) is found in the saliva of man and animals, except carnivora. Human saliva is the most active, next the pig, solipeds slight, ruminants still less, and carnivora very little or none. Ellenberger (2) also believes that in animals it is a specific product of the salivary glands. He states that it attacks raw starch with difficulty but cooked starch easily. On a diet of raw starch (corn) and after masticating the food for one to three minutes, Ellenberger (3) states, sugar can be demonstrated in the oesophagus. He also thinks the action is continued for some time in the stomach.

Meade Smith (4) believes that in ruminants, starch conversion takes place both in the mouth and rumen. Fred Smith (5) does not accept these views and states that the action is very feeble or entirely absent.

Mills (6) states that the saliva of the ox has a very feeble diastatic power. It was therefore the object of this investigation to demonstrate the presence or absence of a diastatic enzyme in the mixed saliva of this animal.

METHOD OF COLLECTING SALIVA

Various drugs have the property of stimulating the secretion of saliva. Chief among these drugs is pilocarpine which, when given subcutaneously in doses of ½ to 1 grain, produces a marked flow of saliva in this animal. It occurred to the writer that pilocarpine would be a very useful agent to employ, as an aid in collecting the saliva, provided it did not in any way influence the diastatic action of the saliva.

Accordingly, preliminary experiments were carried out to determine the action of pilocarpine upon the diastatic action of human saliva, the normal action of this saliva being well understood. It was found that pilocarpine does not inhibit the diastatic action of human saliva, when added to it. Neither does it interfere when taken (via mouth) in large enough doses to cause a marked flow. However, when pilocarpine in aqueous solution is added to a 1 per cent solution of cooked starch and when no saliva is present, it exerts an action similar to that of saliva. Our experiments show that as small an amount as 0.00081 gram will reduce Fehling's solution and when this amount is incubated with 25 cc. of 1 per cent starch, the blue color with iodine disappears in about 20 minutes, and the starch solution will become clear in fifteen minutes, and will show increased reduction of Fehling's solution. It differs from ptyalin in that its action is much slower when used in this amount, but the rate of action can be increased by increasing the amount of the drug. When ½ grain in 25 cc. of distilled water was added to 25 cc. of 1 per cent starch solution, the blue color with iodine disappeared within ten minutes, and the solution was clear in about five minutes. Pilocarpine because of these properties could not be used in our work for stimulating salivary secretion. Enough of the drug can be excreted in the saliva to produce a marked diastatic action which might lead to wrong conclusions.

When ½ grain of pilocarpine is administered subcutaneously to an ox, a marked secretion of saliva occurs in about ten minutes. This saliva contains a small amount of pilocarpine and will produce the characteristic action of this drug upon starch.

Another method of collecting the saliva was worked out. This consisted in stimulating the flow of saliva from glands which had previously

been in a resting state,[1] by irrigating the mouth with water or a weak acetic acid solution, followed by inhalations of strong acetic acid. In addition to these methods, tempting food was sometimes offered which apparently aided in the stimulation of the flow.

The saliva was caught in a clean beaker as it flowed from the mouth. Cattle will often retain the saliva in the mouth and sometimes swallow it, but by holding the head down and inserting the index finger into the mouth at the commissure of the lips, and pulling the commissure downward and outward the retained saliva will flow out. The mechanical stimulation of the clean finger will also help in some cases to induce secretion. Ten cubic centimeters could usually be obtained without much difficulty and quite often much more.

Blood serum which was used in the experiments was obtained by bleeding from the jugular vein. It was obtained by means of a hypodermic needle under aseptic precautions. Twenty to 30 cc. would be drawn into a sterilized flask, and at once placed upon ice. After several hours the serum was drawn off, centrifuged if necessary, and used.

METHODS OF STUDY

Clearing of the starch solution. To three test-tubes of equal size 5 cc. of a 1 per cent cooked Dakomin corn starch solution was added. To the first test tube, 1 cc. of clear or filtered ox saliva was added; to the second, 1 cc. of clear or filtered human saliva (obtained by washing the mouth and stimulating secretion by means of paraffin); and to the third, 1 cc. of distilled water. They were then incubated at room temperature or 40°C. depending upon the series to which they belonged. The three tubes were compared at certain intervals and the amount of clearing recorded.

Iodine tests. At intervals of a few minutes a drop from each of the above test tubes was added to a drop of Lugol's solution. The drops of Lugol's solution were placed upon a glass slab with a white background.

Reduction tests. A series of tubes was made in quadruplicate, similar to those used in the clearing tests. After being incubated for a certain length of time, each was tested with Fehling's solution. The Fehling's solution, alone and also with the cooked starch, was tested for reducing

[1] In one case considerable saliva was being secreted at the time of making the collection, but this saliva showed as strong a diastatic power as any collected from glands which had previously been in a state of rest.

properties. The ox and human saliva was also tested with Fehling's solution.

Serum tests. The experiments using blood serum in place of the saliva were carried out in a similar way.

RESULTS

The results here recorded are based upon the examination of eight different specimens obtained from six different mature bovine animals, of the dairy type. Slight digestion varying in degree was obtained in six examinations, and two were practically negative. The two negative samples were obtained from animals which later gave positive results.

Saliva of the ox is a clear viscid fluid, and when produced in large quantities, it will hang from the mouth in strings. It was always found to be alkaline to litmus.

Our experiments show that the ability of ox saliva to digest cooked starch varies in individuals and even in the same individual at different times. The action produced by 1 cc. of saliva is about equal, in some cases, to the action of four drops of blood serum, in others to about three drops, but when it becomes lower than this, it cannot be detected. Whether the saliva is obtained from glands which have been in an active state, or in a resting state, does not seem to make much difference.

Results of clearing the starch solution. Slight clearing of the starch solution containing 1 cc. of ox saliva could be seen in some cases at fifteen minutes, while in others it took one-half hour before any clearing could be detected. Complete clearing of the solution occurred in about one hour in the majority of cases. Those cases which were not clear after one to one and one-half hours never did become clear. There seemed to be very little, if any difference, in the rate of clearing between the samples incubated at room temperature and those at 40°C. Similar solutions of starch with human saliva and distilled water were used as controls in making the readings. In the case of human saliva the solution became clear in five to ten minutes, while the solution containing water only did not.

Results of iodine test. Although starch solution which contains ox saliva usually becomes clear within one hour, the blue color with iodine remains, nor does it tend to disappear after incubation has been carried on for a period extending over two hours. In the case of the controls, starch with human saliva showed no color with iodine after

about ten minutes, while that of the starch and water continued to give the blue color throughout the incubation period.

Results of the reduction tests. Some cases gave a slight positive reduction at fifteen minutes while others did not, but whenever clearing was marked at fifteen minutes, a slight reduction occurred. If the starch solution was clear at one-half hour, a slight reduction would occur. In the majority of cases the longer the period of incubation, the greater the amount of reduction until the maximum was obtained, which occurred at one and one-half to two hours. Control reduction tests showed starch and human saliva to give a strong reduction after incubation for five minutes. The starch and distilled water tube was tested at the end of the incubation period and was always negative. Human saliva (1 cc.) alone does not reduce Fehling's solution, but 1 cc. of ox saliva will usually give a faint reduction.

Following is the detail of one experiment on the diastase of saliva of the ox.

Animal. Pure bred Guernsey cow. Ten cubic centimeters obtained by the usual methods. Reaction alkaline, glands in state of rest at time of stimulating secretion.

Tube A contains 5 cc. of 1 per cent starch and 1 cc. of ox saliva, made in quadruplicate and labelled A, A^1, A^2, A^3. Tube B contains 5 cc. of 1 per cent starch and 1 cc. of human saliva, made in quadruplicate and labelled B, B^1, B^2, B^3. Tube C contains 5 cc. of 1 per cent starch and 1 cc. of distilled water.

Clearing

NUMBER OF TUBE	TIME IN MINUTES					
	15	30	45	60	90	120
A's...	Slight	Slightly more	Slightly more	Clear		
B's...	Considerable	Clear	Clear	Clear		
C.....	No action	No action	No action	No action	No action	No action

Iodine

NUMBER OF TUBE	TIME IN MINUTES											
	5	10	15	20	25	30	35	40	45	60	90	120
A.....	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
B.....	Blue	No color	No color									
C.....	Blue	Blue				Blue			Blue			Blue

Reduction

NUMBER OF TUBE	TIME IN HOURS			
	½	1	2	2½
A's...	A. Slight reduction.	A^1. Slightly more	A^2. Slightly more	A^3. Same as A^2.
B's...	B. Much reduction.	B^1. Same.	B^2. Same	B^3. Same
C.....				No reduction.

Checks.

1. Fehling's solution plus boiling—negative.
2. Starch and Fehling's plus boiling—negative.
3. Human saliva plus Fehling's plus boiling—negative.
4. Ox saliva plus Fehling's plus boiling—faint trace.

These tests were made with equal amounts of material, carefully measured with a pipette and made in equal sized test tubes. Ten cubic centimeters of Fehling's solution was used in each case.

Details of experiment to show when reduction first occurs. Six tubes each containing 5 cc. of 1 per cent starch and 1 cc. of ox saliva

NUMBER OF TUBE	LENGTH OF INCUB. PERIOD	RESULTS OF REDUCTION TESTS
	minutes	
1	5	No reduction
2	10	No reduction
3	15	No reduction
4	20	Trace
5	25	Slight
6	30	Slight

RESULTS OF BLOOD SERUM EXPERIMENT

There is present in the blood serum of the ox an enzyme which behaves very similarly to the one found in the saliva. In the serum it is much more concentrated and three to four drops of blood serum exerts an action upon starch equal to 1 cc. of saliva. When three to four drops are used, the starch solution becomes clear in one-half to one hour. Reduction of Fehling's solution may occur after fifteen minutes of incubation, but is most pronounced after one to two hours. The blue color with iodine remains throughout.

Detail of series of experiments on the relative diastatic action of ox's serum. Digestion at 40° C.

SERUM SERIES	AMT. OF SERUM USED	AMT. OF STARCH USED	CLEARING						IODINE						REDUCTION				
			15	30	45	60	90	120	5	15	30	45	60	120	15	30	60	90	120
	drops	*cc.*																	
1	2	5	No action	No action	No action	Slight clearing	Slight clearing	Slight clearing	Blue at all times						No Reduct.	No Reduct.	No Reduct.	No Reduct.	No Reduct.
2	3	5	No action	No action	No action	Slight clearing	Slight clearing	Slight clearing	Blue at all times						No Reduct.	Trace	Considerab.e	Slight	Considerable
3	4	5	Slight clearing	Clearer	Clearer	Clear	Clear	Clear	Blue at all times						Slight	Slight	Considerable	Considerable	Considerable
4	6	5	Quite clear	Clearer	Clear	Clear	Clear	Clear	Blue at all times						Considerable	Considerable	Much	Much	Much
5	8	5	Quite clear	Clearer	Clear	Clear			Blue at all times						Much	Much	Much	Much	Much
6	15	5	Quite clear	Clear	Clear	Clear			Blue at all times										Much

1. Two drops of serum and Fehling's solution.—No reduction.
2. Three drops of serum and Fehling's solution—faint trace of reduction.
3. Four drops of serum and Fehling's solution—trace of reduction.
4. Six drops of serum and Fehling's solution—trace of reduction.
5. Eight drops of serum and Fehling's solution—trace of reduction.
6. Fehling's solution plus boiling—negative.
7. Fehling's solution plus starch, 5 cc. at 40° C. for two hours—negative.

THERMAL DEATH POINT

It was found in one series of experiments that heating the enzyme found in the serum to 63°C. for one minute will greatly inhibit the action of the enzyme, and heating for one minute at 65°C. will practically destroy its activity.

NUMBER OF TUBE	DEGREE OF HEAT FOR 1 MIN.	LENGTH OF INCUB.	CLEARING		REDUCTION
			½ hr.	1 hr.	
		hour			
1	No heat	1	Clear	Clear	Much
2	63°	1	Almost clear	Clear	Good reduction, not as much as in 1
3	65°	1	No action		Slight trace
4	75°	1	No action		Slight trace
5	84°	1	No action		Slight trace
6	90°	1	No action		Slight trace
7	Boil	1	No action		No reduction

Ten drops of serum used.
Ten drops, plus Fehling's—Slight trace.

DISCUSSION

This starch-splitting enzyme or enzymes found in the saliva of the ox acts much slower than the starch-splitting enzymes found in human saliva. It is present in very minute traces and in some samples of saliva cannot be demonstrated. This enzyme or enzymes is much more concentrated in the blood serum and is very likely carried into the saliva, along with other salivary constituents, which accounts for the small amount present in the saliva.

Owing to the nature of the food which this animal takes, one could naturally expect the serum to contain a relatively large amount of diastatic ferments, and it would only be natural for some of these ferments to be excreted in the saliva.

Carlson and Ryan (5) found a similar condition existing in the cat. They found that the saliva of the cat contains a starch splitting ferment. This ferment is not a specific product of the salivary glands but the diastase of the blood carried into the saliva along with other salivary constituents. They also found the starch splitting enzyme, or enzymes, to be very much more concentrated in the cats' serum than in the cats' saliva.

Because of the slowness of action of this ferment in the saliva of the ox and the small amount present, it is doubtful if it has any digestive value. It would seem that if nature intended the saliva of the ox should contain a starch splitting enzyme, it would be present in larger quantities and be more rapid in its action, and that it would resemble ptyalin of man in these respects.

SUMMARY

It is recognized that the number of observations is small for drawing definite conclusions, but that the uniformity of results points strongly to the conclusions drawn.

1. Saliva of the ox contains a starch splitting enzyme or enzymes, which varies in amount in individuals, and at different times in the same individual.

2. This enzyme or enzymes is not a specific constituent of the saliva but comes from the blood which contains similar enzymes in much higher concentration.

3. These enzymes have a thermal death point at about 65°C. when heated at this temperature for one minute.

4. This enzyme or enzymes is unimportant as far as its digestive ability is concerned.

I wish to express my great appreciation to Dr. C. J. V. Pettibone for his constant interest and advice.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS[1]

IV. The Effect of Thyroid Secretion on the Pressor Action of Adrenin

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The employment of organ extracts to determine the physiological effects of the specific secretions of endocrine glands has, in a number of instances, led to much confusion and contradiction. Schäfer (1) has recently emphasized the fact that "we are not justified in assuming that because we obtain evidence of the presence of active substances in such extracts that they are necessarily preformed in the organ and still less that they are passed out from it into the blood." The widely different effects produced on the circulation by intravenous injection of variously prepared thyroid extracts afford a striking example of the uncertainty of a method in which an impure product is used. Thus, whereas some investigators have observed a rise in blood pressure, others report a fall, while still a third group finds no change whatsoever. Similarly discordant observations are recorded with regard to changes in pulse rate. Unless an active principle in pure form is available, as is the case in the secretory product of the adrenal medulla, it would seem desirable, where possible, to study the physiological effects of an internal secretion by stimulating to activity the gland producing it.

The recent work of Cannon and Cattell (2), reported in a previous paper of this series, has placed the secretory innervation of the thyroid on a firm basis. These observers have employed as their criterion of secretory activity the electrical change giving rise to the current of action. They have demonstrated that an action current is produced in the thyroid gland by stimulation of the cervical sympathetic nerve or by injection of minute amounts of the sympatheticomimetic sub-

[1] The earlier papers of this series were published in this Journal, 1916 (July), xli, pp. 39, 58 and 74.

stance, adrenin (3). The gland secretes promptly on stimulation, i.e., an action current is obtained after a latent period of only a few seconds. No evidence of secretory activity is observed on stimulating the vagus nerves nor is there any electrical change after injection of the vagotropic drug, pilocarpine.

As a result of these studies, two methods are at hand for inducing secretory activity in the thyroid gland. It is the object of the present communication to present evidence of the presence of thyroid secretion in the circulating blood after stimulation of the cervical sympathetic nerves or after injection of adrenin and to demonstrate that thyroid secretion increases the effectiveness of adrenin in raising arterial pressure. Its effects on blood pressure and pulse rate are also recorded.

PREVIOUS WORK ON THE EFFECT OF THYROID SECRETION ON THE PRESSOR ACTION OF ADRENIN

Having been led to believe from the work of certain Viennese investigators (4) that thyroid and adrenal glands mutually stimulate one another, Asher and Flack (5), in 1910, set out to determine whether during stimulation of the laryngeal nerves, which they regarded as the secretory nerves of the thyroid, a given dose of adrenin would produce a greater circulatory response than before. They demonstrated to their own satisfaction that the rise in blood pressure during stimulation was higher and of longer duration than during a previous control period and felt justified in concluding that (1) stimulation of the laryngeal nerves causes secretion of the thyroid gland; (2) thyroid secretion sensitizes sympathetic endings to the action of adrenin. These results were obtained fairly consistently in rabbits, but inconstantly in cats. Their tracings are far from convincing, since the differences in the height of the curves before and after stimulation amount to but a few millimeters, and are not more than might reasonably occur during variations in the depth of anesthesia or from respiratory fluctuations. Aside from this criticism, Cannon and Cattell (6) have shown that the laryngeal nerves, as branches of the vagi, are in no way directly concerned with secretory discharge in the thyroid gland.

In a brief preliminary report recently published, Oswald (7) records a series of observations on the effect of injection of iodthyreoglobulin on the circulation. It produces no alteration in blood pressure or pulse rate. But after intravenous injection of this substance, which Oswald calls the true secretory product of the thyroid, adrenin causes a rise in pressure which is higher and of longer duration than before.

The curve may, in fact, be twice as high and twice as long (no tracings are reproduced.) This effect is manifest, however, only after a short latent period and persists for a considerable time, having been demonstrated after the lapse of 1½ hours. It is Oswald's belief, therefore, that iodthyreoglobulin renders sympathetic endings more sensitive to the action of adrenin and he suggests that both latent period and long duration of effect are probably dependent on the colloidal nature of his product.

These studies are suggestive. But Asher and Flack, according to most recent evidence, did not stimulate the true secretory nerves of the thyroid and Oswald injected a glandular preparation the identity of which with thyroid secretion was an assumption.

METHODS OF THE PRESENT INVESTIGATION

Cats were used in all the experiments. The animals were prepared essentially according to the method described by Elliott (8) for use in his physiological quantitation of adrenin solutions. After preliminary ether anesthesia, a tracheal cannula was inserted. Care was taken not to injure the large vein running along the trachea, since it carries in large part the venous return from both thyroid glands. To destroy the brain, a perforation was made in the back of the orbit and a probe was passed through it into the spinal canal beyond the level of the medulla. Artificial respiration, maintained by a motor-driven, air-blast interrupter was now begun. The depth and rate of respiration must be carefully adjusted at the start if the animal is to be kept in good condition for a number of hours and these factors should not be altered during the course of an experiment. Both vagi were cut. The cervical sympathetic nerves were dissected free from the vagus sheaths for 2 or 3 cm., ligated low in the neck and severed distal to the ligatures. The depressor, when present as a separate nerve, was also cut. The probe was now again inserted through the orbit and the cord thoroughly destroyed to about mid-thorax, a level corresponding to the third or fourth thoracic segment. Usually in the course of twenty minutes or half an hour the blood pressure fell to a constant level between 25 and 50 mm. Hg. An electric heating pad placed beneath the animal permitted the maintenance of a normal or nearly normal temperature.

Medium sized or small cats (2 to 3kg.) were found more suitable than larger animals. Males were used almost entirely in order to avoid the variable conditions in the female genital organs. In cats

pithed as described, the resulting rise in blood pressure after an injection of adrenin varies directly with the amount injected. Vasomotor and respiratory fluctuations are abolished. In the words of Elliott, "the circulatory system responds with the accuracy of a chemical balance to any dose of adrenin." In certain instances, despite repeated pithing, irregular and wavy curves were obtained; it was found best under these conditions to reject the animal and make a fresh preparation.

A cannula inserted into the femoral artery was connected with a mercury manometer, sodium carbonate being used as anticoagulant. Injections of adrenin were made through another cannula placed in the femoral vein on the opposite side. A slowly revolving kymograph was set so that the speed was uniform in all experiments.

The adrenin employed for injection was the commercial "Adrenalin Chloride" of Parke, Davis and Company, and was fresh. Immediately before each experiment it was diluted, usually 1: 100,000, with distilled water, in which the adrenin remains without obvious chemical change for a considerable period. The injections were made from a syringe with 1 cc. barrel, graduated to tenths and fiftieths of a cubic centimeter. A metronome, beating at intervals of one second, permitted delivery of the dose uniformly in successive injections. Usually 0.2 or 0.3 cc. were injected at the rate of 0.02 cc. per second.

For stimulating the cervical sympathetic nerves a tetanizing current, rhythmically interrupted at the rate of 24 times a minute, was employed, the nerve being laid over a platinum electrode supported by a clamp. The current was of such strength that it could comfortably be borne on the tip of the tongue and always caused maximal pupillary dilatation when the side with intact eye was stimulated.

A time marker registering 30-second intervals served also as base line for zero blood pressure and as signal.

Any deviation from the usual technique will be described in its proper place.

PRESENTATION OF RESULTS

Effects of sympathetic stimulation. In all of the experiments the first two or three injections served as controls.[2]

[2] Due probably to incomplete filling of cannula and vein with the adrenin solution at the start, the first curve was often smaller than those made a few minutes later (see, for example, fig. 2), and has at times therefore been disregarded in the presentation of control tracings.

In figure 1, curve *C* shows the blood pressure record during the period of stimulation. The slight rise beginning and ending promptly with the stimulus is not to be regarded as specific, since a similar tracing obtains with thyroids removed. Moreover, by ligating the large vessels in the neck on the stimulated side, the rise can be almost entirely abolished, indicating its vasomotor origin. It is significant, in view of the discordant statements mentioned earlier in the paper, that there is no appreciable effect on blood pressure during sympathetic stimulation, since, as will be pointed out later, throughout this time the thyroid is pouring out its secretion.

The height of the adrenin rise immediately after stimulation and even 35 minutes later (see fig. 1, *D*, and fig. 2) is no higher than that obtained in the controls. It is only after a *latent period*, in this instance about 50 minutes, that the increased effectiveness of adrenin is manifest. The latent period may vary from about 40 to 60 minutes, but is always observed. The effect of the thyroid secretion, when once its activity is evident in augmenting the circulatory response, increases in degree usually for several hours, and may persist for as long as 7 hours (see figs. 11 and 12.)

In Experiment XII (figs. 1 and 2) the maximum effect was observed some 100 minutes after stimulation, when the rise obtained was 88 per cent higher than in the control. Considerably greater increases may occur. It is of interest that the ratio between the height of the original rise and that produced after stimulation represents precisely the increase in the efficacy of a given dose of adrenin. For example, if 0.3 cc. (1: 100,000) causes before stimulationa rise of 25 mm. Hg., and produces two hours after stimulation a rise of 75 mm. Hg., injection of 0.1 cc. of adrenin will accurately reproduce (within 2 or 3 mm. Hg.) the original curve at this later time.

There is a distinct variability in different animals in the magnitude of the effect resulting from stimulation. Several cats with "snuffies" and a few lean, scrawny animals which had recently been shipped to the laboratory in crates from a distance showed remarkably little increase in pressor response.

Tracings taken on a more rapidly revolving drum have shown that neither during nor after stimulation is there any significant alteration in pulse rate. A considerable acceleration of heart rate accompanies each adrenin injection. A series of observations was recorded to determine if, coincident with the augmented efficacy of adrenin in raising arterial pressure, there was also a greater pulse-rate increase at the height of the rise. Table 1 indicates that such an increase does not

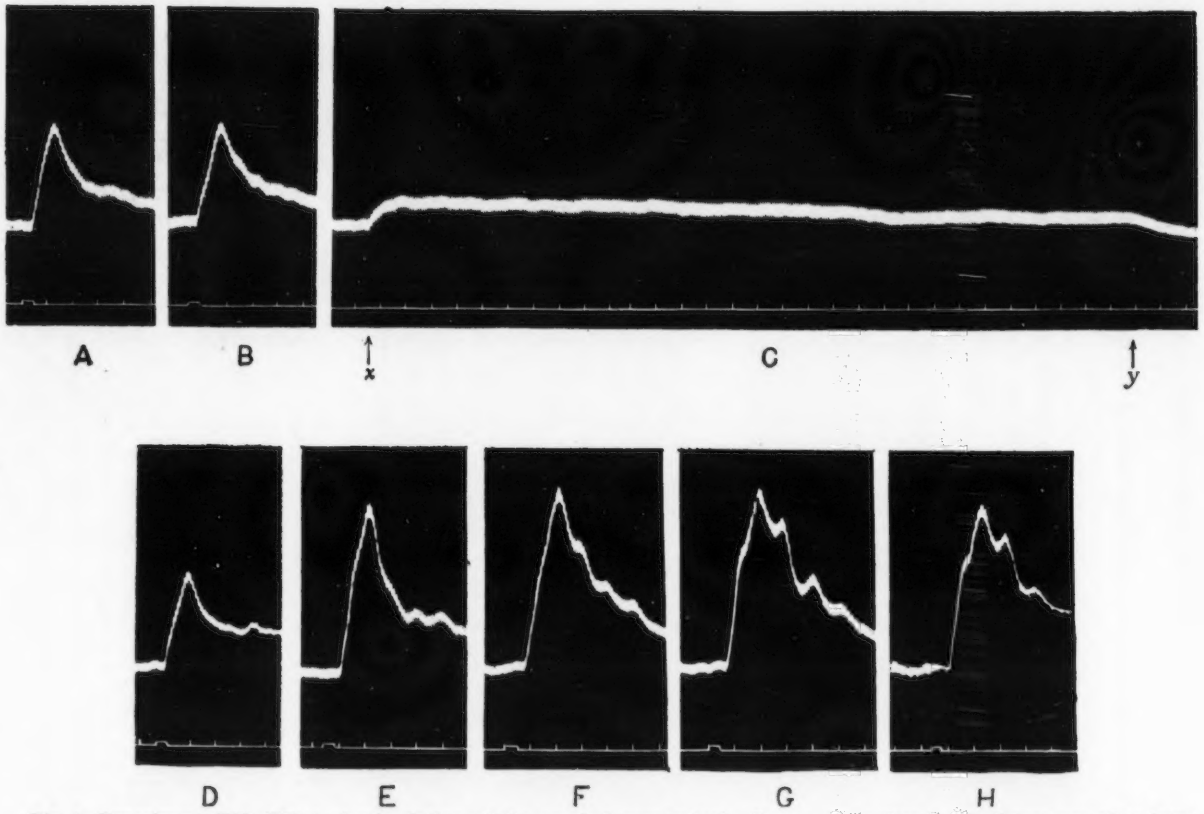

Fig. 1. Experiment XII. Effect of stimulation of the cervical sympathetic. Curves show rises in blood pressure after intravenous injection of 0.3 cc. adrenin, 1: 100,000. In this, as in all subsequent tracings, base line records time in half minutes and is at zero blood pressure level. The notches in the base line mark the periods of adrenin injections. All injections were made at the rate of 0.02 cc. a second. *A* and *B*, controls, taken 8 minutes apart. *C* (12 minutes after *B*), stimulation of left cervical sympathetic nerve for 15 minutes (beginning at *x* and ending at *y*). *D*, 3 minutes after stimulation. *E*, 53 minutes after stimulation. *F*, 93 minutes after stimulation. *G*, 133 minutes after stimulation. *H*, 168 minutes after stimulation.

occur, and serves also as an illustration of a typical protocol (abbreviated):

TABLE 1.

Effects of sympathetic stimulation

Experiment LIX. June 24. Small, female cat.

10.10 Ether. Struggled vigorously.

10.15–10.30 Tracheotomy. Pithed through medulla. Vagi cut. Sympathetics dissected and ligated.

10.35 Pithed to mid-thorax. On warming pad.

		RISE IN BLOOD PRESSURE (MM. HG.)	PULSE BEFORE INJECTION	PULSE AT HEIGHT OF RISE	TEMPERATURE
11.15	Injection No. 1. 0.3 cc. adrenin, 1: 100,000 at rate of 0.02 cc. per second.	34	153	225	37
11.23	No. 2.....	35	156	213	
11.28–11.33	Stimulation of left cervical sympathetic.				
11.45	No. 3.....	30	162	216	37
11.55	No. 4.....	34	165	213	
12.10	No. 5.....	42	165	216	
12.50	No. 9.....	50	171	222	37.5
12.57–1.02	Stimulation of right cervical sympathetic				
1.05	No. 10.....	55	171	225	
1.30	No. 12.....	61	171	213	
2.30	No. 15.....	70	162	214	
2.40	No. 16.....	70	165	212	37.5

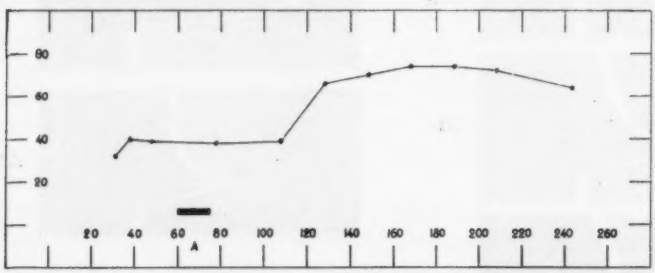

Fig. 2. Complete chart of Experiment XII. (See fig. 1.) Here, as in all other charts, ordinates represent blood pressure in mm. Hg., abscissae time in minutes. The dots (joined by solid lines) denote the height of the blood pressure rise resulting from injection of the specified amount of adrenin. At *A*, stimulation of the left cervical sympathetic nerve for 15 minutes.

Effect of injecting a large dose of adrenin. Adrenin mimics sympathetic impulses, and Cannon and Cattell, after its injection, have demonstrated an action current in the thyroid. Figures 3 and 4 (Experiment XVIII) picture the results after injecting 10 cc. of adrenin, 1: 100,000. There is the usual latent period followed by gradual augmentation in pressor response until, 160 minutes after the injection,

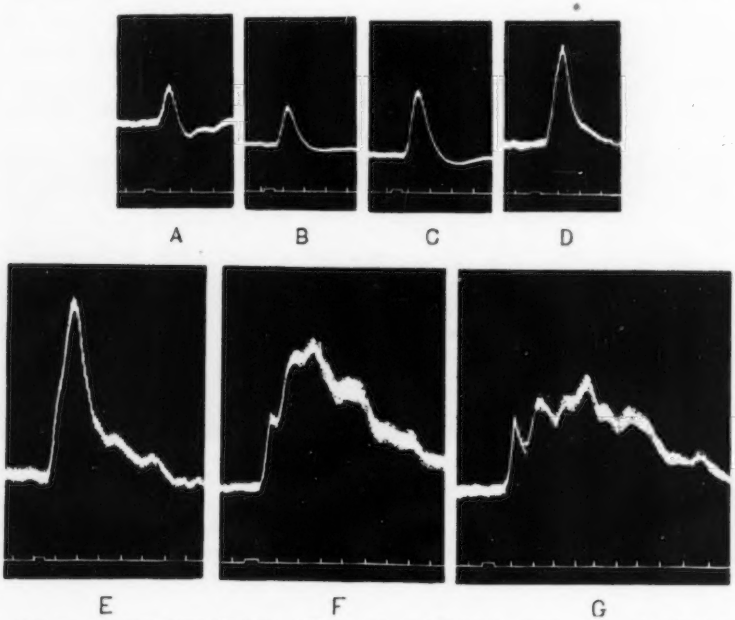

Fig. 3. Experiment XVIII. Effect of injecting a large dose of adrenin. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (13 minutes after *A*, injection of 10 cc. adrenin, 1: 100,000, during a 10-minute period). *B*, 32 minutes after the injection. *C*, 60 minutes after the injection. *D*, 102 minutes after the injection. *E*, 162 minutes after the injection. *F*, 327 minutes after the injection.

the rise produced is 82 mm. Hg. as compared to the control of 18 mm. Hg.—an increase in effectiveness of over 300 per cent! The removal of both thyroids at *B*, figure 4, did not prevent the effect from continuing for a number of hours, though as seen in figures 1, 2, 3 and 4 there is at times a tendency for the curve to diminish toward the end of the experiment.

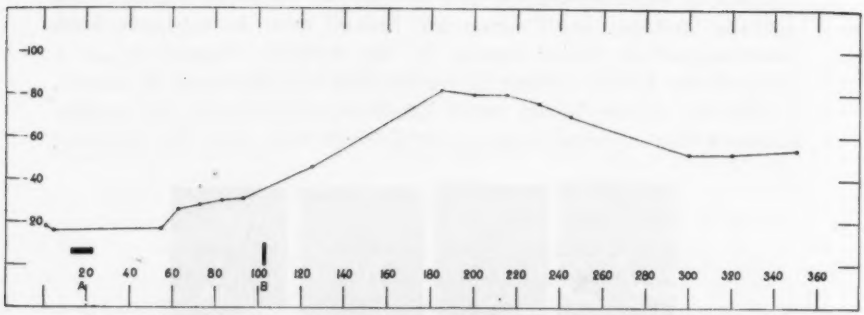

Fig. 4. Complete chart of Experiment XVIII., (See fig. 3.) At *A*, injection of 10 cc. adrenin, 1: 100,000, during a 10-minute period. At *B*, both thyroid glands removed.

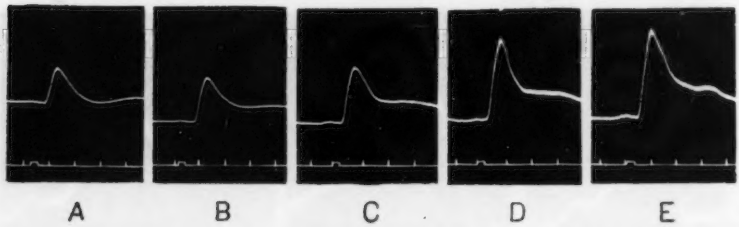

Fig. 5. Experiment XIII. Effect of repeated injections of minute doses of adrenin. Curves show rises in blood pressure after injection of 0.2 cc. adrenin, 1: 100,000. *A*, control. *B*, 35 minutes after *A*. *C*, 100 minutes after *A*. *D*, 120 minutes after *A*. *E*, 135 minutes after *A*.

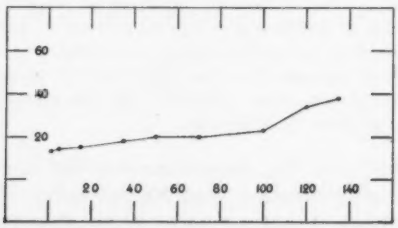

Fig. 6. Complete Chart of Experiment XIII. (See fig. 5.)

Effect of repeated injections of minute doses of adrenin. Repeated testing of the circulatory response requires the repeated injection of small doses of adrenin at intervals; and the electrical evidence of Cannon and Cattell indicates that even minute doses are sufficient to induce secretory activity in the thyroid. In Experiment XIII, (figs. 5 and 6) 9 injections of 0.2 cc. adrenin, 1:100,000, were made in the course of 135 minutes. Again there is seen the gradual gain in the height of the rise, from a control of 14 mm. Hg. to a response of 38 mm. Hg., an increase of over 160 per cent.

A few of the cats struggled vigorously while being placed in the holder and during the stage of primary anesthesia. They took the ether

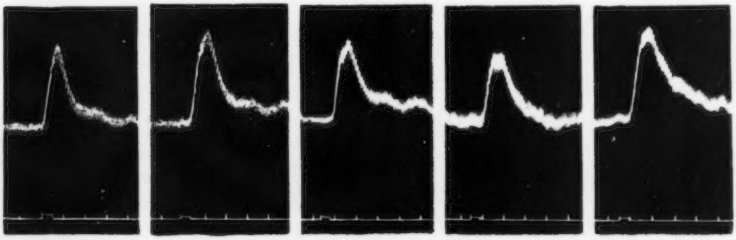

Fig. 7. Experiment LX. Failure of effect of cervical sympathetic stimulation after removal of both thyroid glands. Double thyroidectomy performed 1 hour and 45 minutes prior to first adrenin injection. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (8 minutes after *A*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes.) *B*, 2 minutes after stimulation. *C*, 31 minutes after stimulation. *D*, 86 minutes after stimulation. *E*, 131 minutes after stimulation.

poorly. Their tail-hairs stood on end and the pupils became widely dilated. It was noted on beginning to take control records some 40 or 50 minutes later that there was a progressive increase in the height of the three curves, though taken at 5 or 10 minutes intervals. Emotion (9), ether (10) and asphyxia (11), all conducive to adrenal discharge, had apparently caused sufficient liberation of adrenin to stimulate the thyroid. It is probable that there was also direct nervous stimulation of the thyroid through impulses passing along the cervical sympathetic. The 40 or 50 minutes required to prepare the animal for the experiment had served as the necessary latent period and the thyroid secretion at once manifested its presence.

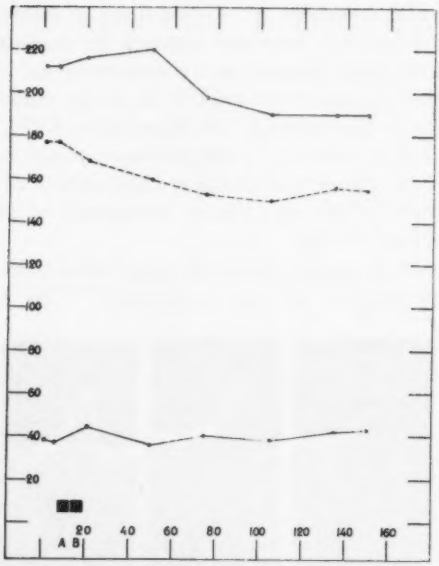

Fig. 8. Complete chart of Experiment LX. (See fig. 7.) Dots, joined by solid lines (lower curve), represent rises in blood pressure as in other charts. Dots, joined by interrupted lines (middle curve), represent pulse rate before injection of the standard dose of adrenin. Circles, joined by solid lines (upper curve), represent pulse rate at the height of the adrenin response. At *A*, stimulation of the left cervical sympathetic for 5 minutes; at *B*, stimulation of the right cervical sympathetic for 5 minutes.

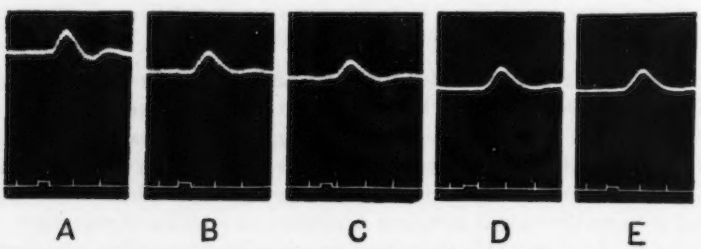

Fig. 9. Experiment XIX. Failure of effect of injecting a large dose of adrenin after removal of both thyroid glands. Double thyroidectomy performed 1 hour and 10 minutes prior to first adrenin injection. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (20 minutes after *A*, injection of 5 cc. adrenin, 1: 100,000, during a 5-minute period.) *B*, 15 minutes after the injection. *C*, 35 minutes after the injection. *D*, 60 minutes after the injection. *E*, 80 minutes after the injection (and 105 minutes after *A*).

Failure of increased pressor effects after removal of both thyroid glands. Evidence has been presented to show that stimulation of the cervical sympathetic or adrenin injection, with thyroid glands intact, causes increased efficacy of adrenin in raising arterial pressure. In figures 7 and 8 (Experiment LX) and figures 9 and 10 (Experiment XIX) are shown the results in animals in which both thyroid glands had been removed a short time before beginning the experiment. It will be noted that, *in the absence of the thyroids, neither stimulation of the cervical sympathetic nerves nor adrenin injection is effectual in producing an increase in pressor response.*

In the cat, thyroids and parathyroids are so closely related anatomically that it is rarely possible to remove either set separately. With one exception, thyroidectomy in these experiments has meant thyroparathyroidectomy. In this single instance it was possible, after thyroid removal, to leave one lower parathyroid with intact blood-supply. The result differed in no respect from that obtained when all four glands were removed. Hoskins and Wheelon (12), moreover, have shown that parathyroid removal tends to increase rather than decrease sympathetic irritability. So that it may be safely assumed that parathyroids exert no appreciable effect on the results under discussion.

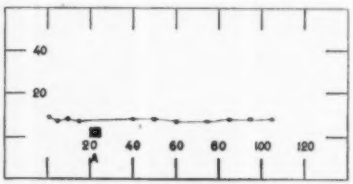

Fig. 10. Complete chart of Experiment XIX. (See fig. 9). At *A*, injection of 5 cc. adrenin, 1: 100,000, during a 5-minute period.

Sympathetic stimulation followed by immediate removal of both thyroid glands. The latent period necessary for thyroid secretion to give evidence of its presence might be explained in one of two ways: (1) it may take a considerable period after stimulation for secretion to begin; or (2) the secretion, though promptly liberated, may require this time either for becoming active itself or for initiating some other process in the body which causes the observed effects. The matter is easily decided. In Experiment XL (figs. 11 and 12) stimulation of both cervical sympathetics was followed by immediate removal of both thyroid glands. The result justifies the conclusion, already suggested by the rapid electrical response observed by Cannon and Cattell, that there is a prompt liberation of thyroid secretion following sympathetic stimulation.

Sympathetic stimulation after removal of the adrenal glands. It has

been reported by numerous observers (Krause, Fraenkel, Trendelenburg, Ott and Scott and others) that in clinical states of hyperthyroidism, as well as after injection into animals of thyroid extract, there is an increase in the adrenin content of the blood and the assumption has therefore been made that thyroid secretion acts as a direct stimulant to the suprarenal capsules causing them to yield adrenin to the blood in large quantities. It is suggestive also that in cats with experimental hyperthyroidism Cannon, Binger and Fitz (13) found the adrenal glands to be nearly three times their normal size.

It might, therefore, be inferred that the increased effect of adrenin in raising blood pressure after inducing secretion in the thyroid is due

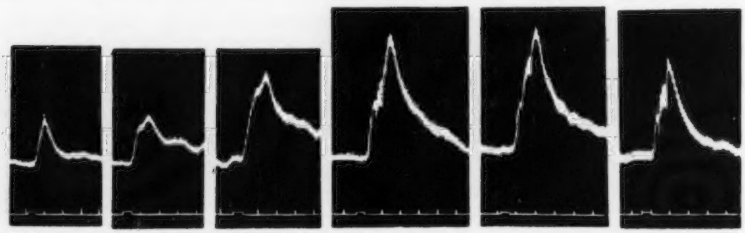

Fig. 11. Experiment XL. Effect of sympathetic stimulation followed by immediate removal of both thyroid glands. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (14 minutes after *A*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes. Five minutes later, double thyroidectomy.) *B*, 35 minutes after stimulation. *C*, 125 minutes after stimulation. *D*, 210 minutes after stimulation. *E*, 360 minutes after stimulation. *F*, 465 minutes after stimulation.

to a greater amount of circulating adrenin which would make the injected dose more efficacious. Against this explanation, to be sure, is the absence of a rise in pressure after stimulation, the invariable latent period, in spite of the fact that both thyroids and adrenals secrete promptly, and the fact that none of these conditions are consistent with a gradually increasing effect.

The final proof is brought in Experiment XLIV, (figs. 13 and 14) in which a double adrenalectomy through a median incision[3] was

[3] It was found feasible to pith only brain and medulla in animals on which laparotomy was to be performed, since this operation on cats pithed to mid-thorax invariably caused a dangerous or fatal fall in pressure. Failure to destroy

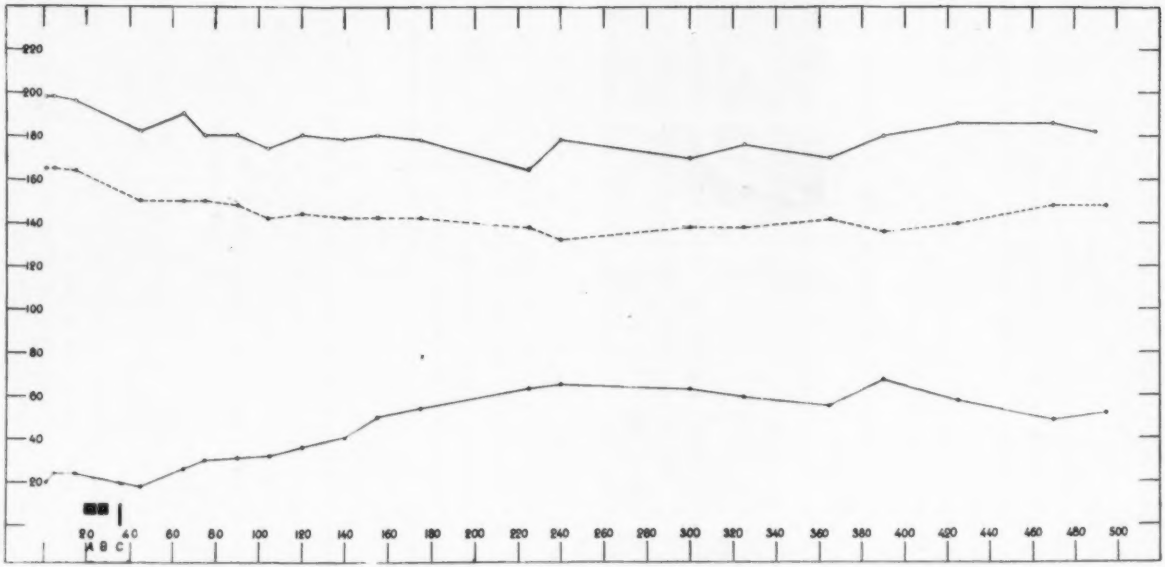

Fig. 12. Complete chart of Experiment XL. (See fig. 11.) Dots, joined by solid lines (lower curve), represent rises in blood pressure, as in other charts. Dots, joined by interrupted lines (middle curve), represent pulse rate before injection of the standard dose of adrenin. Circles, joined by solid lines (upper curve), represent pulse rate at the height of the adrenin response. At *A*, stimulation of the left cervical sympathetic for 5 minutes; at *B*, stimulation of the right cervical sympathetic for 5 minutes. At *C*, double thyroidectomy.

performed 50 minutes prior to the first adrenin injection. The absence of the ordinary latent period is not surprising, since Crowe and Wislocki (14) have pointed out that any operative procedure on either adrenal liberates sufficient adrenin to produce glycosuria and this amount would undoubtedly suffice to initiate secretory activity in the thyroid. The increase in the size of successive curves prevails as in the unoperated animals.

In other similar experiments in which the adrenal veins were more quickly ligated on entering the abdomen, the usual latent period was observed.

Effects of injecting the crystalline, iodin-containing compound isolated from the thyroid by Kendall (15). Through the kindness of Dr. E. C. Kendall of the Mayo Clinic, it has been possible to experiment with this substance, which, in minute doses has produced strikingly beneficial results in cretins and myxedematous individuals (16). A solution containing 0.3 mg. per cubic centimeter of the crystalline compound and having an iodin content of 0.184 mg. per cubic centimeter (60 per cent) was employed. The dosage used was approximately 1 mg. of the crystals per kg. of body weight.

Intravenous injection into previously thyroidectomized cats caused no appreciable alteration in blood pressure or pulse rate but did produce an increase in the effectiveness of adrenin as a pressor agent, as in the stimulation experiments. (See table 2.) Two points are worthy of special note; first, there was no marked latent period after injection of Kendall's compound, the effect being demonstrable a few minutes after its introduction into the circulation; second, the duration of the effect was relatively shorter than in the experiments in which the thyroid gland was stimulated in the animal body.[4]

Critical discussion. Kepinow (17) and others have demonstrated that after injection of extracts of pituitary, there is an increase in the

subsidiary vasomotor centers probably accounts for the greater variations in the basal blood-pressure levels in these experiments. Such variations are of no significance, however, since Asher (Hering-Festschrift, 1910, Hager, Bonn) has shown that the effect of vasomotor stimulation is the same whether the initial blood pressure be high or low.

[4] As has been mentioned earlier in this paper, Oswald has observed both latent period and long duration of effect after injecting iodthyreoglobulin and has explained them both on the basis of the colloidal nature of his product. It is conceivable that this explanation of these phenomena holds good also with regard to the secretion liberated by the gland in the body. The more rapid manifestation of activity and shorter effective period of Kendall's preparation may possibly be dependent on its crystalline character.

TABLE 2.

Effects after injection of Kendall's crystalline, iodin-containing thyroid compound

Experiment LVIII. June 23. Small, female cat. Weight, 1.9 kg.

9.30–9.42 Ether. Thyroidectomy (complete). Pithed through medulla. Both vago-sympathetic trunks cut. Pithed to mid-thorax. On warming pad.

		RISE IN BLOOD PRESSURE (MM. HG.)	PULSE BEFORE INJECTION	PULSE AT HEIGHT OF RISE
10.20	Injection No. 1. 0.3 cc. adrenin, 1: 100,000, at rate of 0.02 cc. per second.	48	144	201
10.25	No. 2	51	147	207
10.35	No. 3	52	148	207
10.52–10.55	5 cc. compound, intravenously			
11.00	No. 5	62	156	198
11.25	No. 7	74	159	198
12.20	No. 11	72	156	195
12.30–12.33	5 cc. compound, intravenously			
12.35	No. 12	96	153	198
12.50	No. 13	92	162	207
2.20	No. 16	54	164	204
3.05	No. 18	45	141	184

power of adrenin to raise arterial pressure. According to Weed, Cushing and Jacobson (18) the pituitary gland secretes actively on stimulation of the superior cervical sympathetic ganglion. Pituitary secretion, however, cannot play any rôle in modifying the results of the experiments herein described. For the upper central nervous system and its circulation are so thoroughly destroyed by pithing that there can be no appreciable absorption from the subarachnoid space. And incontrovertible is the fact that with the thyroid glands removed, though other conditions remain unchanged, the usual effect is absent.

Börner has pointed out that in rabbits, pituitary extract slows heart rate and causes diminished cardiac systole, and attributes the increased effectiveness of adrenin, after pituitrin injection, to its greater concentration in the blood as a result of mechanical slowing of the circulation. A similar criticism is not applicable to the present work. For, (1) at the time of increase in pressor response there is no slowing of heart rate; (see fig. 12 and table 1.); (2) there is no progressive lengthening of latent period between adrenin injection and circulatory

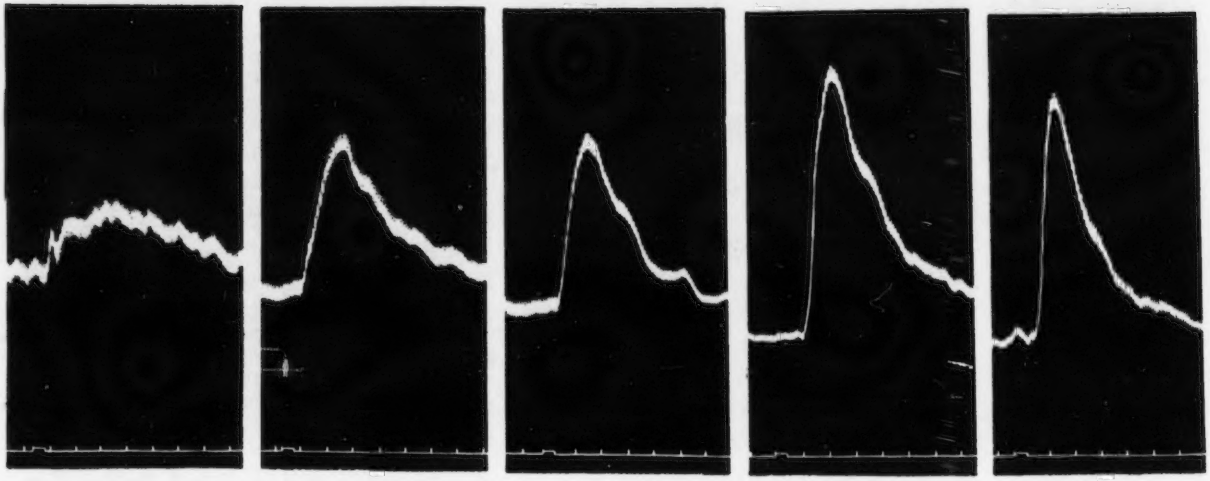

Fig. 13. Effect of cervical sympathetic stimulation after removal of the adrenal glands. Both adrenal glands removed through median incision 50 minutes prior to first adrenin injection. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1:100,000. *A*, control. *B*, 55 minutes after A. *C*, 180 minutes after *A*. (5 minutes after *C*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes.) *D*, 325 minutes after *A*, and 130 minutes after stimulation. *E*, 400 minutes after *A*, and 205 minutes after stimulation.

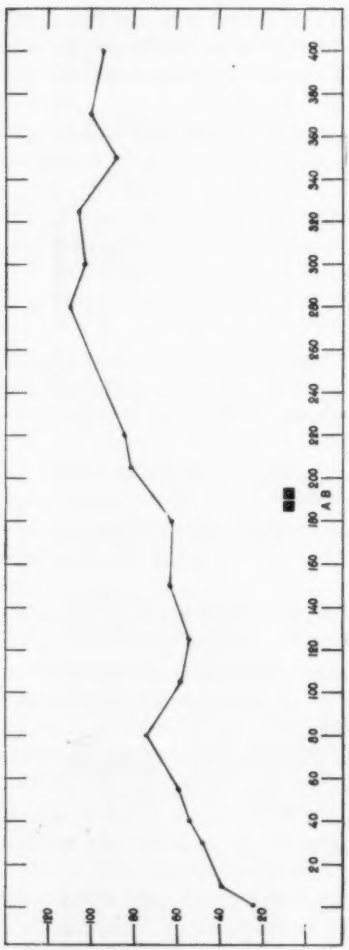

Fig. 14. Complete chart of Experiment XLIV. (See fig. 13.) At *A*, stimulation of left cervical sympathetic for 5 minutes. At *B*, stimulation of right cervical sympathetic for 5 minutes.

response as would occur if the circulation were slowed; (3) pulse pressure tends often to increase rather than diminish; (4) in a number of experiments the increase in the height of the curves has been followed by a subsequent diminution in their size (see figs. 1, 2, 3, 4, 11 and 12); and finally, (5) with thyroids removed, and therefore no increase in adrenin effectiveness, there may be a slowing of pulse rate during the course of the experiment (see fig. 8).

It is clear therefore, that the greater adrenin response after thyroid stimulation is due to a sensitization of the sympathetic structures acted on by adrenin in raising blood pressure. The action of thyroid secretion on sympathetic endings is a selective one, for there is no concomitant increase in excitability of the augmentors of the heart.

SUMMARY

1. In cats, after stimulation of the cervical sympathetic in the neck, there can be demonstrated an increase of the effectiveness of adrenin in raising arterial pressure. This increase may be as much as 200 to 300 per cent.

2. Injection of adrenin, even in minute amounts, produces a similar effect.

3. This effect is manifest only after a latent period, which may vary from about 40 to 60 minutes. There is then a progressive increase in the height of the curves, the maximum being reached in from 2 to 3 hours after stimulation. The effect is of considerable duration, having been observed for as long as 7 hours.

4. When the thyroid glands have been previously removed, cervical sympathetic stimulation or adrenin injection does not produce an increase in the pressor response to adrenin. It is therefore justifiable to conclude that (a) stimulation of the cervical sympathetic or adrenin injection induces secretory activity in the thyroid gland, (thus confirming the observations of Cannon and Cattell); and (b) thyroid secretion renders more excitable the sympathetic structures acted on by adrenin in raising arterial pressure.

5. Stimulation of the cervical sympathetic causes the thyroid to secrete promptly, since thyroidectomy immediately after stimulation is followed by a progressive augmentation in pressor response similar to that observed when the glands are left in the animal throughout the course of the experiment.

6. Intravenous injection into thyroidectomized cats of a solution of the crystalline, iodin-containing compound isolated from the thyroid by Kendall, is followed by an increase in the efficacy of adrenin as a pressor agent. The effect is demonstrable almost immediately after injection and is of relatively shorter duration than in the stimulation experiments. This substance causes no appreciable alteration in blood pressure or pulse rate.

7. Neither during nor after stimulation of the thyroid gland through its secretory nerves is there any significant change in blood pressure or pulse rate.

8. After thyroid stimulation, even at the time when adrenin is more effective as a pressor agent, there is no increase in the augmentation of pulse rate produced by adrenin injection. This indicates a selective action of thyroid secretion in sensitizing sympathetic tissues to the action of adrenin, since the increase in vascular response is not associated with greater excitability of the augmentors of the heart.

9. In cats whose adrenal glands have been previously removed, there can be demonstrated, after thyroid stimulation, an increase in pressor response similar to that seen in unoperated animals. Though there is some evidence in the literature indicating that thyroid secretion acts as a secretory stimulant to the adrenals, the increased effectiveness of adrenin as a pressor agent after thyroid stimulation is not dependent on a greater amount of circulating adrenin.

It is a pleasure to express my cordial thanks to Dr. W. B. Cannon for his helpful interest and advice.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

I. Volume Changes and Venous Discharge in the Limb

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In several papers from this laboratory the relation of various endocrine glands and particularly the suprarenals to the vasomotor system has been considered. In these studies the vasomotor apparatus was considered as a whole. The fact, however, that various components of the vasomotor system react differently to adrenin makes it desirable to investigate further the effects of that substance on vascular conditions throughout the body. This is in effect to determine the influence of adrenin on the distribution of the blood. Such studies are needed in case of various glands on account of their possible broad significance in endocrine biology. Theoretically any hormone might exercise a profound influence upon body functions by shifting the circulation in favor of one organ at the expense of another. This possibility has received little attention at the hands of investigators.

So far as we are aware the only investigators who have studied the specific problem of the relation of the suprarenals to the distribution of the blood are Falta and Priestly (1). Their method was to administer to an animal subcutaneously a large dose of adrenin (1 mgm. per kilo) and then from one to several hours later, when the animal was supposedly under the influence of the drug, to note the condition of various organs. This was done by opening the body, quickly clamping off the organs and observing the degree of anemia or hyperemia present. It was reported that the adrenin injections resulted in an accumulation of blood in the lungs, brain, liver and kidneys, while the skin, mucous membranes, spleen and muscles were pale. The conclusion was reached that the function of the suprarenals is so to regulate the blood vessel caliber throughout the body that an adequate circulation to the vital tissues can be maintained with a minimal cardiac effort.

This work is open to various objections. Whether subcutaneous injections of adrenin at all closely simulate adrenal discharge is doubtful. Kleiner and Meltzer (2) have found that so far as glycosuric effects at any rate are concerned the results of introducing adrenin into a vein and under the skin are notably different. Also mere inspection of the organs is but a rough way of determining their blood content and might well fail to disclose significant conditions. That the method was inadequate is indicated by the failure of the reported results to agree in some particulars with more adequate plethysmograph studies. For example there is no doubt that the liver and kidneys contract under the influence of adrenin although they were reported by Falta and Priestly as hyperemic.

The best known and most complete investigation of the effects of adrenin on the blood content of different organs is the first, made by Oliver and Schaefer (3) in 1894–1895. The reports of this work contain most of the pertinent data that have ever been recorded on the subjects treated. The work still needs supplementing, however, in certain respects. For example no determinations of changes of gut volume were made. Also their method of administering the extracts used was ordinarily by short lasting injections whereas normal adrenal discharge supposedly persists through a longer period of time. This persistence might permit secondary adjustments to occur and significantly alter the reaction picture. The possible effects of variations in dosage also failed to receive much attention, the supposition at that time being that such variations produce merely quantitative differences in the reactions. But investigations of more recent years have shown that the effects of adrenin may be diametrically different depending upon the amount used. For example the pulmonary vessels are dilated by small doses but constricted by large (4). In the respects mentioned the subject is still open to investigation.

The first studies of the effects of adrenin on limb volumes were reported in Oliver and Schaefer's paper just mentioned. It was found that the limb usually contracted. (In the literature they have been misquoted on this point.) In some of their experiments, however, an expansion of the limb was noted. These results have been confirmed by Vincent (5). It was found that the expansion was particularly likely to occur toward the end of an experiment. It was regarded as "passive." In certain instances one limb expanded while its fellow contracted. This inconstancy was ascribed to a struggle between active constriction of the arterioles and dilatation of the larger vessels within the plethysmograph.

Several investigators have recorded observations on the effects of adrenin on the outflow from perfused limbs. In the use of the Läwen-Trendelenburg perfusion method of assaying adrenin abundant proof has been secured that this substance in all dilutions causes vasoconstriction in the legs of the frog (6). Similar results have been reported by Brodie and Dixon (7), Embden and v. Fürth (8) and by Biedl (9).

Gerhardt (10) dissected out the vessels of a bit of living muscle and studied them under a microscope. When adrenin was injected he was not able to detect changes in the caliber of either the arterioles or the venules but concluded that the arterioles as a matter of fact must have constricted for otherwise the veins would have shown a dilatation as a result of the increased arterial pressure that ensued.

The foregoing observations have been interpreted as indicating that adrenin causes vasoconstriction in skeletal muscle. But the part played by the skin in such experiments has not received adequate consideration. That adrenin causes vasoconstriction in the cutaneous vessels has been reported by Velich (11), Baum (12), Elliott (13) and Vincent (14), From the study of the limb as a whole, therefore, without knowing the extent to which the skin participated in the reaction no conclusion is justified regarding the reaction in the muscle.

Hartman (15) has recently investigated the relative effects of adrenin in the "peripheral" and the splanchnic circulations. The superior and inferior mesenteric arteries, the coeliac axis and sometimes the renal arteries were tied off. Under those conditions it was noted that a dose of adrenin that had previously given a pressor reaction now gave purely or predominantly a fall of pressure. In other experiments the carotid and subclavian arteries and the abdominal aorta above the iliac were tied off. The circulation was thereby restricted largely to the trunk and thoracic viscera and the splanchnic region. Under such conditions the circulation was regarded as predominantly splanchnic. (That this was so follows from the work of Burton-Opitz (16) who found that the liver and kidneys receive a large proportion of the blood passing through the aorta.) Hartman found that when the peripheral circulation was so excluded the dosage of adrenin that normally gave predominantly a fall of pressure now gave a rise. In four cases the two types of experiment were alternated in the same animal. In each instance clamping the peripheral arteries transformed a depressor to a pressor reaction. The results of clamping the splanchnic vessels were less consistent but in general left unchanged or augmented the depressor effect. From these observations it would appear that a discharge of

adrenin into the blood stream would result in a simultaneous constriction in the splanchnic and dilatation in the peripheral circulation. Since in these experiments the "peripheral circulation" included a large area of skin the vessels of which react by constriction it follows that the vessels of the muscles must have dilated. No experiments were made to determine to what extent the skin and muscle actually did participate in the reactions.

That a specific relationship exists between muscular efficiency and circulating adrenin has been asserted by several investigators. Oliver and Schaefer (17) showed in 1894 that suprarenal extracts increase the activity of skeletal muscle both in mammals and frogs. Dessy and Grandis (18), Panella (19) and Joteyko (20) have reported that adrenin improves the activity of amphibian muscle. According to Joteyko the effect is due not to stimulation of the muscle but to a change in the sarcoplasm that renders it more sensitive to nervous impulses. In amphibian muscle the effect is very slight.

In mammals the beneficial influence of adrenin is more marked and more easily demonstrated. Cannon and Nice (21) have shown that injections of the drug or stimulation of the suprarenal glands through their nerves improves the activity of stimulated muscle, sometimes 100 per cent. This favorable effect was noted even when the blood pressure was mechanically prevented from rising. Gruber (22) has found that adrenin greatly hastens the recovery of a muscle from fatigue. This result he concluded was not due to any influence on the vasomotor system whereby the circulation of the muscle was increased. No direct studies were made, however of the effects of adrenin on the circulation of the muscle under the conditions of his experiments.

As to how the beneficial influence of adrenin on muscle is brought about several possibilities present themselves. Among these are increased mobilization of dextrose, a direct influence of the adrenin on muscular metabolism and changes in the distribution of the blood favorable to the muscle. It was thought that further investigation might throw some light on this problem.

In this and subsequent papers will be reported the results of a series of studies of the effects of adrenin on the volume of various organs and on the venous outflow from them. The first structure to be considered is the limb or, more specifically, muscle and skin.

Methods. In all cases dogs were used as experimental animals. They were anesthetized with ether or ether and morphine.

The plethysmograph used for the leg was of the familiar Mosso type.

It was simply a glass cylinder of appropriate diameter closed at one end by a paraffined cork stopper through which passed an outlet tube. The other end was adapted to the leg by a thin rubber cuff. Leakage under the cuff was prevented by a liberal use of vaseline. Air transmission was employed. The outlet tube was connected to a recorder by rubber tubing in which was interpolated a glass T-tube. Through this the system was put under a positive pressure amounting to 4 or 5 cm. of water.

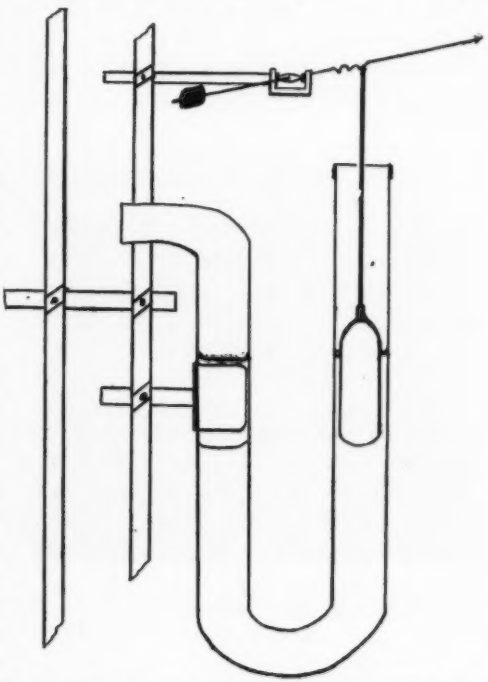

Fig. 1. Float recorder. (Diagrammatic; writing lever shown at reduced magnification.)

After considerable experimentation with different types of recorders that described below was improvised and adopted for routine use. It consists essentially of a water manometer and float and a counterpoised "heart lever" to which the movements of the float are transmitted. In the form first used the transmission was by means of an aluminium wire as shown in figure 1. Later a thread was substituted for the wire and found to be more satisfactory. The thread is attached to the float by means of a bit of colophonium wax. For a float an empty adrenalin ampoule serves admirably. Experience showed that the best results are secured when the bore of the manometer is only slightly greater than the outside diameter of the float. The instrument proved to have several points of superiority. As compared to a water manometer in which the thrust is transmitted directly to the writing point this has two advantages: The record can be written at any magnification

desired simply by adjusting the attachment of the transmission thread along the arm of the writing lever; also, the lateral extension of the writing lever provides considerable latitude for adjustment of the apparatus to the drum whereas when the direct thrust is transmitted as in the familiar mercury manometer it is difficult to arrange for two or more records in the same ordinate. As compared with a piston recorder of equal delicacy this has the advantage of requiring no lubrication. Also a bit of dust or distortion of the transmission from accurate alignment does not bring the apparatus to a standstill. The small cost, simplicity and ease of securing the apparatus are other obvious advantages.

Injections or infusions were made into a femoral vein through a cannula. The cannula was connected by a rubber tube with a reservoir of isosmotic saline solution. The tube was closed by a clip near the cannula. The drug was introduced into the tube and flushed instantaneously into the vein by opening the clip or it was infused more slowly from a burette through a hypodermic needle introduced through the rubber tube into the venous cannula.

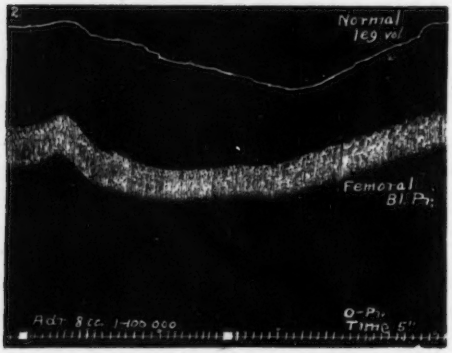

Fig. 2. Normal leg contracting under the influence of adrenin, depressor infusion. (Reduced to ½.)

As an index to the general efficiency of the dosages of adrenin employed arterial blood pressure was always recorded. In most cases the pressure was measured with a mercury manometer damped to exclude the pulse oscillations. These were recorded by means of a small tambour connecting with a side branch of the tube leading to the artery. This combined method, as pointed out by Sollman and Pilcher, has advantages over the use of either manometer or tambour alone.

Effects of adrenin in the intact limb. The effects of adrenin on the volume of the intact limb were first investigated. Varying dosages were used from those which gave a fall of pressure to those causing a marked rise. The duration of administration varied from about one

second to ten minutes. Blood pressure from the femoral artery was recorded by the method previously described. Ordinarily the leg used in the plethysmograph was a forelimb but a hind limb also was occasionally employed. Dogs of about 14 kilograms weight were selected. The legs of these could be included in the plethysmograph up to the lower end of the humerus or femur.

Figure 2 shows the effect of a relatively small dose of adrenin administered by infusion. Eight cubic centimeters of 1–100,000 solution was introduced during a period of one hundred and thirty seconds. The leg volume at first increased slightly, the expansion lasting about

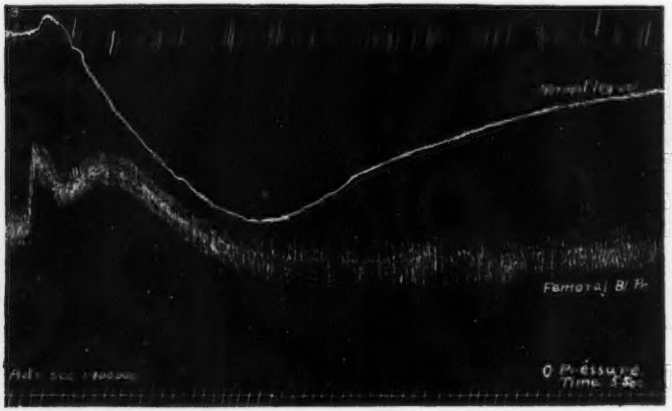

Fig. 3. Normal leg contracting under the influence of adrenin, pressor injection. (Reduced to ½.)

thirty seconds, coinciding with a brief rise of arterial pressure. Then as blood pressure fell the volume decreased. The lowest point was reached, however, eighty-five seconds after blood pressure started to rise or thirty-five seconds after the infusion was discontinued. The blood pressure and leg volume reached their normal at the same time. This graph standing alone would probably be taken to indicate that the volume changes in the limb were passive. In many cases, however, such an interpretation is not possible.

Figure 3 for instance shows a case in which an injection of adrenin caused a marked contraction of the leg which was clearly not a passive

effect since the greater part of the contraction occurred while the blood pressure was above normal.

Figure 4 shows one of the cases that were occasionally seen in which an expansion instead of a contraction resulted from adrenin. The case is striking in that only a small instantaneous injection was used. The expansion in this case again is obviously not a passive effect; the limb did not begin to increase in volume until the pressor reaction was passed and it continued for some time after the pressure had reached essentially its normal level. In the light of later experience it is suspected that this was an unusually muscular dog but at the time the record was made the significance of that condition was not realized and no note on the point was made. Oliver and Schaefer reported that dilatation of the limb is most likely to occur toward the end of an experiment. In the case shown in figure 4 the experiment was just beginning.

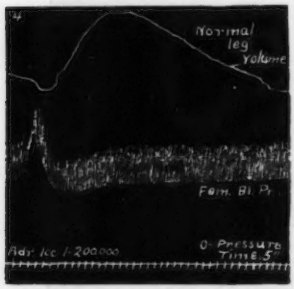

Fig. 4. Normal leg expanding under the influence of adrenin. (Reduced to ½.)

The results of this series of experiments as a whole were not unlike those of Oliver and Schaefer. In all 156 experiments were made in twenty-five different dogs. Seventeen dogs showed no other effect than contraction at any stage of the experiment or with any dosage. One showed expansion of the limb only while seven showed sometimes one and sometimes the other depending upon the dosage or a change in the condition of the animal during the course of the experiment. The conditions that could bring about a reversal in the volume reactions were not fully worked out but it was noted that hydremic plethora is one of them.

In most instances the effects of adrenin in the limb could not be interpreted as passive. There was little parallelism between the blood pressure and limb volume reactions. One experiment was particularly instructive in this respect. The dog was given a slow adrenin infusion lasting ten minutes. This resulted in a brief elevation of pressure followed by a return to normal and persistence at the normal level or slightly below normal during the whole of the infusion period. Both leg and spleen volumes were recorded. In both a sharply marked contraction occurred which lasted throughout the infusion period and promptly ceased immediately after the infusion was stopped.

One passive phenomenon only was noted with any degree of regularity; the leg volume usually was increased for a short time during the first pressor stage of the vasomotor reaction. This expansion according to our records is to be interpreted as due to the first effect of the adrenin in the heart. After it enters the aorta the drug is at once carried through the coronary circulation and stimulates the muscle augmenting the cardiac output while the peripheral resistance remains unaffected. This produces a brief initial increase in blood pressure which in turn causes a passive expansion of various organs at the expense of the great veins. In this expansion the limbs share. Then as the drug reaches the periphery the local effects come into play and give the volume curve its characteristic alterations. This feature is illustrated in figures 2 and 3. (In figure 3 the volume recorder was writing slightly in advance of the blood pressure manometer.)

Effects of adrenin in the skinned limb. The next step in the investigation was to determine whether and if so to what extent the limb contraction was due to vasoconstriction in the muscle. The components of the limb that have chiefly to be considered in such determination are the bone, muscle and skin. As to the bone its fixed volume precludes its taking any part, directly, at least, in the contractions or expansions of the limb. To determine the relative parts played by the muscle and skin the obvious method was followed of recording first the volume changes with the limb intact and then removing the skin and repeating the experiment.

The skin was incised all around just above the lower end of the humerus. It was then removed by traction aided by the occasional use of a scalpel. At the carpal articulation the paw was amputated. Ordinarily but few vessels had to be ligated. Oozing from the end of the stump was prevented by a tourniquet applied as a figure 8, the strands crossing between the ulna and radius. Despite the severity of the procedure the dogs usually showed little or no evidence of shock.

The results of removing the skin were striking and, on the whole, consistent. Under all conditions of dosage, duration of administration and resultant effects on blood pressure adrenin caused expansion of the skinned leg. One dog only offered an exception to this rule and that only with large doses. In all in this series one hundred and thirty-five injections or infusions were made in eighteen dogs. In some instances the leg was skinned after the reaction had been determined with it in its normal state. In other cases the experiment was begun with the skin removed. The experiment was also varied by re-

cording simultaneously the volume changes in two legs, one being skinned and the other normal. The results were the same whatever method was used. Figure 5 illustrates a typical case. The first graph was taken with the leg in a normal condition. The skin was then removed and the adrenin injection repeated. The volume reaction was reversed by removing the skin. The animal in this case showed more shock than usual but a similar reversal occurred when the operation resulted in no apparent shock or when the changes in a normal and a skinned leg were recorded simultaneously. In different experiments a

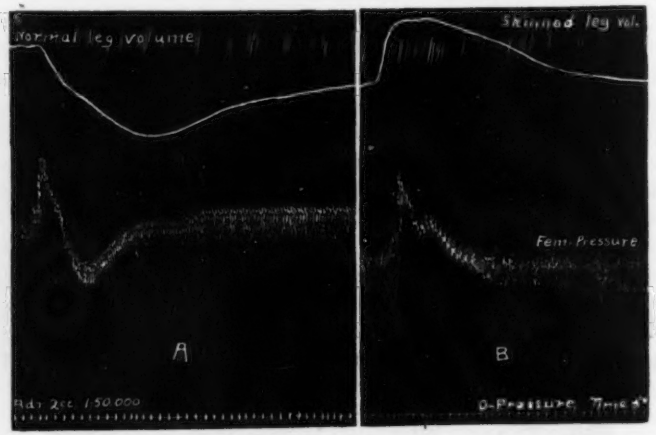

Fig. 5. *A*, Normal leg contracting; *B*, same leg after removal of the skin expanding under the influence of adrenin. (Reduced to ½.)

persistance of the limb expansion after the blood pressure had returned to normal indicates that the dilatation was not a passive process.

The objection might be offered that the trauma incident to removing the skin had induced a local vasomotor paresis which would account for the expansions observed. This objection could probably be met by removing the skin from the distal part of the leg leaving a considerable part of its circulation intact, taking a record with this skin wrapped about the limb and then repeating the injection with the skin left outside the plethysmograph. Indeed such an experiment was planned. But the results obtained in the experiments next to be described rendered this superfluous.

Throughout this series it was observed that removal of the skin from the leg caused it to expand under the influence of adrenin whereas the normal limb ordinarily contracts. The contraction therefore would seem to be due to vasoconstriction in the skin while the expansion in the skinned leg indicates that adrenin causes vasodilatation in the muscle. One's first impression is likely to be that the relative mass of the skin as compared with that of the muscle of the limb is too slight to permit vasoconstriction of the cutaneous vessels to overcompensate for vasodilatation in the muscles. As a matter of fact however, in the types of dogs used in these experiments the mass of the muscles in the

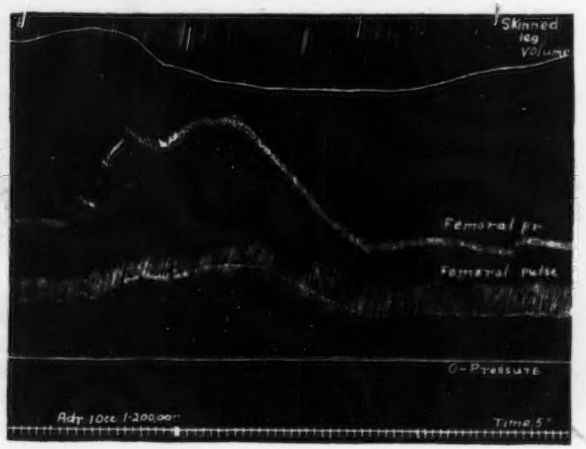

Fig. 6. Skinned leg contracting under the influence of adrenin, pressor infusion. (Reduced to ½.)

lower segments of the legs was relatively small as compared with that of either the bone or skin. It is quite probable that with short legged unusually muscular dogs the vasodilatation would so predominate as to cause an expansion of the limb. A similar result would probably be obtained if it were feasible so to adjust the plethysmograph as to include the muscles of the proximal segment of the limb.

In case of one dog an exception to the ordinary effect was noted. This is illustrated in figure 6. Ten cubic centimeters of adrenin 1–200,000 was introduced during a period of eighty seconds. During the first half minute the volume of the skinned leg increased. Then a

decrease occurred which persisted for some time after the blood pressure had returned to normal. The cause of the contraction in the absence of the skin was not determined. Possibly it might be ascribed to vasoconstriction in the bone such as Drinker and Drinker (23) have recently observed in perfusion experiments, the constriction holding back sufficient blood from the peripheral veins to cause a decrease in the total mass within the plethysmograph.

Effects of adrenin on venous outflow. Earlier in the paper mention was made of the work of different observers who found that adrenin

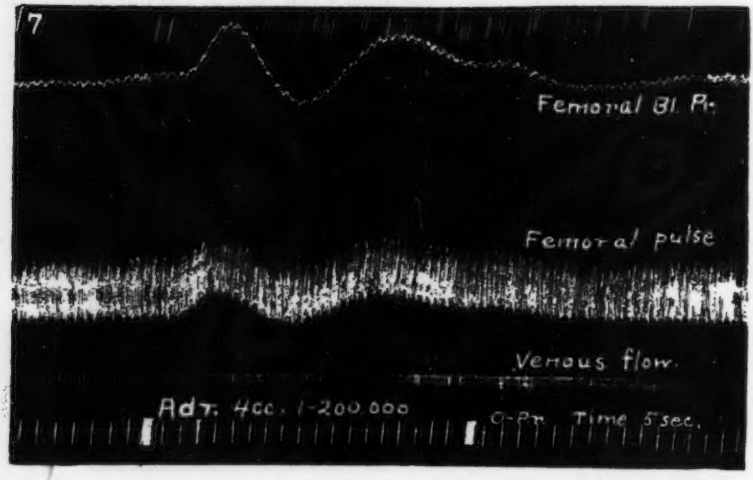

Fig. 7. Outflow from a vein of a muscle augmented by adrenin, pressor infusion. Registered by drops. No reduction.

perfused through the limbs decreased the rate of outflow. This was noted by Biedl (9) in the dog, the animal used in our experiments. Experiments on isolated limbs, however, tell little as to what would be the effect of an adrenin discharge in an intact animal. The effects of adrenin on the blood flow in the limb under normal conditions would depend upon the resulting arterial and venous pressures as well as upon local effects in the tissues involved. Furthermore, perfusion experiments made on the limb as a whole do not determine to what extent the changes of outflow depend upon effects in the skin, muscle or bone. As to the effects of adrenin on the bone circulation in the intact animal

apparently nothing is known. Recent work by Drinker and Drinker (23) has shown that when it is perfused through the isolated bone the rate is decreased.

Capps and Mathews (24) have shown that adrenin injections of the magnitude used in our experiments have little or no effect on venous blood pressure. Measuring the rate of outflow from an open vein, therefore, under various conditions of adrenin administration should afford a reliable index of the effects of such administration on the blood flow in an intact animal.

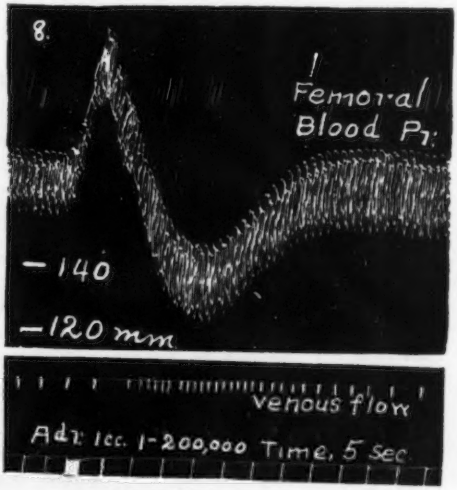

Fig. 8. Outflow from a vein of a muscle augmented during the depressor phase of reaction to adrenin. Registered by drops. No reduction.

The researches were accordingly extended in the following way: A femoral vein was exposed in the groin. One of its branches coming from an underlying muscle was further isolated and in it an oiled cannula was tied. The outflow was recorded by drops on a kymograph drum by means of a key and signal magnet. At the same time arterial pressure was recorded as in the earlier experiments of the series. Adrenin was administered through the femoral vein of the opposite leg both by instantaneous injections and by infusions at various rates. Then a cutaneous branch of the same femoral vein was cannulated and the experiments repeated. The conditions therefore, were in every way comparable except that the outflow in one case was from the muscle and in the other from the skin.

Effect in muscular branches. Figures 7 and 8 show the results secured in case of the muscular branches. The rate of outflow was increased, sometimes slightly and sometimes more than 100 per cent. That the effect was active rather than passive is shown by both figures. In figure 7 the outflow continued at an increased rate for half a minute after the blood pressure had returned to normal. In the case shown in

figure 8 an augmented outflow and a lowered arterial pressure occurred simultaneously. Indeed the reduced arterial pressure in such cases is probably due very largely to exactly this augmented outflow from the vessels of the muscles. Similar results were obtained throughout the series both with large and small doses. It is evident, therefore, that adrenin exercises a selective vasodilator effect in skeletal muscle.

Effect in cutaneous branches. The effect in the skin is exactly the opposite of that in muscles. Figure 9 shows a typical result when the cannula is inserted in a cutaneous branch. After a momentary acceleration of the flow, due supposedly to the propulsive effect of contraction of the peripheral vessels the outflow is markedly decreased. Infusion experiments showed that the retardation persisted as long as the administration of adrenin was continued and for about half a minute thereafter. Here again the fact that the venous outflow was retarded irrespective of the changes of arterial pressure shows that the effects were not passive. In no case was adrenin observed to cause an augmented outflow from the cutaneous vessels. A source of error that not infrequently gave results that might be interpreted as due to vasoconstriction both in the muscular and cutaneous branches was clotting of the blood in the cannulas. In all cases in which the rate of flow failed to return approximately to normal when the adrenin was discontinued the records were discarded.

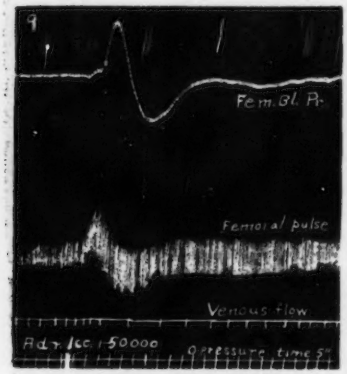

Fig. 9. Outflow from a cutaneous vein decreased by adrenin injection. Registered by drops. (Reduced to ⅔.)

DISCUSSION

From the foregoing observations it is obvious that in an intact animal adrenin in any general sense is neither a vasoconstricting nor a vasodilating agent. It is one or the other depending upon what vessels are involved. In some cases, as in the lungs, the effect depends also upon the dosage (4). Neither can one generalize to the effect that the splanchnic vessels are constricted while the peripheral vessels are

dilated. In the peripheral circulation are included not only the skin but also other less extensive structures that respond by constriction while observations not yet reported have shown that the gut is often dilated. The effect of adrenin in such a structure as a limb is a composite one, the limb as a whole either contracting or expanding depending upon whether vasodilatation in the muscle or vasoconstriction in the skin predominates. The fact that the muscular tissues form the greatest mass in the trunk and limbs would seem to account for the fact that adrenin passed through the extra-splanchnic circulation causes a fall in arterial pressure.

It is probable that the selective vasodilator effect of adrenin in skeletal muscle accounts to a considerable degree for its favorable influence in improving contractile activity as well as hastening recovery from fatigue.

This selective vasodilator effect may account for certain other phenomena otherwise difficult of explanation that have been described. The waves of augmented activity that appear in ergographic curves when the experiments are carried to unpleasant lengths may possibly be due to adrenal discharges. The same explanation is more plausible in case of a phenemenon observed by Hill and Nabarro (25). They measured the outflow from the femoral vein of a dog during an epileptic fit and found it several times greater than during rest. Periods of excitement are known to be accompanied by augmented adrenal discharge and this is probably true of the type of excitement studied by Hill and Nabarro.

SUMMARY AND CONCLUSIONS

1. Adrenin injected by vein in the intact animal generally causes in all dosages a constriction of the limbs. Occasionally, however, expansion occurs.

2. Removing the skin converts the contraction to expansion.

3. Adrenin in both pressor and depressor dosages causes an increase in the venous outflow from muscles.

4. Adrenin, therefore, causes active vasodilatation in the muscles.

5. Adrenin causes decreased outflow from the skin hence vasoconstriction in the cutaneous vessels.

6. The effects of infusions and of instantaneous injections are qualitatively similar.

7. The local vasodilator effects of adrenin probably account for at least a part of its beneficial effect in active and fatigued muscle.

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THE EFFECT OF PITUITARY EXTRACT ON THE FROG'S ESOPHAGUS

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The physiology and to a limited extent the pharmacology of the frog's esophagus have been investigated by various experimentalists chief among whom may be mentioned Botazzi and Gruenbaum (1) and Stiles (2). The former studied sections of the organ suspended in a moist chamber and found the longitudinal strata to be rhythmically active and to respond to drugs essentially like the mammalian intestine. Stiles on the other hand studied the transverse musculature of the opposite extremities of the esophagus suspended in Ringer's fluid and observed that, in comparison with one another, the pharyngeal end had a more active rhythm but a less extensive amplitude than the gastric.

Relative to the effect of pituitary extract on the gastro-intestinal system of mammalia, an extensive literature has accumulated during the past decade (4). Among other phenomena, our attention has been several times called to the presence of a depressor substance whose effect is to markedly decrease the tone and rhythmic activity of the intestines of the rabbit and the dog. The chief students of this depressor action are:

1. Bayer and Peter (3) who showed that certain preparations of pituitary body (they used principally the "Pituitrin" of Parke, Davis and Company) decreased the tone and the contractions of the rabbit's intestine.

2. Shamoff (4) who showed that this depression of the excised rabbit's intestine was produced by most of the extracts available to him, but not by Armour's pituitary liquid. He did not use "Pituitrin."

(3) Hoskins (5) who has called attention to this depressor action on the dog's intestine in situ and thinks that the present commercial preparations vary in the content of depressor substance, the variation probably resulting from differences in the mode of production.

As this experimental work was done during the early months of summer, it may be of importance to make a brief statement in regard to the care and condition of the frogs from which the tissue was taken. The frogs were kept out of doors in a large tank provided with an earth covered platform placed just above the surface of the water and shielded from the direct rays of the sun by a canvass covering. They were fed on earth worms; fresh water was admitted once or twice per day; about once per week a little salt and lime were added to the water. The whole batch remained in excellent condition throughout the entire period covered by the experiments.

I used, in these experiments, sections of the freshly excised esophagus taken from pithed frogs and suspended in oxygenated Ringer's Fluid. These sections were as follows: (1) Longitudinal from the whole length of the organ; (2) rings from near the pharynx and from near the stomach; and (3) longitudinal segments from near the pharynx and from near the stomach.

The method of suspending and registering was similar to that used by Stiles. One end of the section was fastened below to a glass rod bent so as to dip into a small conical "tissue-bath" which itself was surrounded by a water jacket equipped with a constant temperature regulator. The free end of the section was attached by means of a silk thread to a light lever which was adjusted to write on a smoked surface. All preparations of tissue were heavily weighted to prevent the appearance of troublesome tone changes.

The preparations of pituitary extract employed were Armour's pituitary liquid and Parke, Davis and Company's pituitrin. These were used in strengths of from 1:125 to 1:300. This would be in terms of the fresh gland substance from 1:9000 to 1:22,000 (6), approximately, which is very much more dilute than that used by Bayer and Peter on the rabbit's intestine.

1. *Longitudinal sections of the whole esophagus.* Application of pituitary extract was followed by a decrease in tone, rhythm, and amplitude of contraction. In most cases, the contractions ceased entirely for from one to three minutes and then resumed with a more rapid rhythm but less extensive amplitude. The tone did not tend to be restored during the continuance of the drug in the tissue-bath, though the amplitude of the contractions sometimes approximated the normal.

Application of adrenaline, which affects the frog's esophagus as it does the rabbit's intestine, deepened the depression produced by

pituitary extract. Pilocarpine and barium chloride both counteracted the depression.

The tissue did not seem to suffer any permanent injury from contact with the drug, for the tone and contractions became normal almost immediately after fresh fluid had been put into the tissue-bath. Repetition, now, of the application of the extract was followed by the same series of events as followed the initial use of the drug.

2. *Portions of the opposite ends of the esophagus.* Having observed the above recorded effects on longitudinal sections of the whole esophagus, it seemed important in view of the difference in rhythmicity at the opposite extremities of the organ (Stiles) to determine whether pituitary extract would show differences as regards its effect at the two ends of the organ. Accordingly, sections from the opposite extremities were compared with one another, (a) circular gastric and pharyngeal and (b) longitudinal gastric and pharyngeal. In making the comparisons, the sections were suspended in separate baths but preferably in the same bath where conditions would be identical.

(a) The circular muscle of both the gastric and pharyngeal ends was depressed. The depression of the pharyngeal end was tardily produced as compared with that of the gastric, the latter responding almost instantaneously with decrease in tone, rhythm, and extent of contraction, the former chiefly with decrease in tone.

(b) The longitudinal muscle of both the gastric and pharyngeal ends was depressed. The depression at the gastric end was very marked, while that at the pharyngeal was only slight.

3. *Influence of temperature.* Portions of the organ were also tried at different temperatures between 22°C. and 38°C. While as has been shown by previous investigators the tissue is more active at certain temperatures than at others, the reaction to pituitary extract was not altered, the tissue responding to the drug proportionately as actively at low as at high.

4. *Influence of oxygen.* It has been stated by certain authors that the depressor action may be augmented in the presence of oxygen due to the ease with which the pressor substance oxidizes. In view of this, experiments were performed in which the supply of oxygen was cut off completely. Of course, under these circumstances, the tissue tended to lose tone and to be less active than when oxygen was supplied, but on the whole the reaction to pituitary extract was unchanged and restoring the supply of oxygen produced no effect other than could be attributed to the oxygenation (see fig. 1).

Fig. 1. Whole esophagus, showing the effect of pituitary extract on the longitudinal muscle deprived of oxygen. Time, one minute.

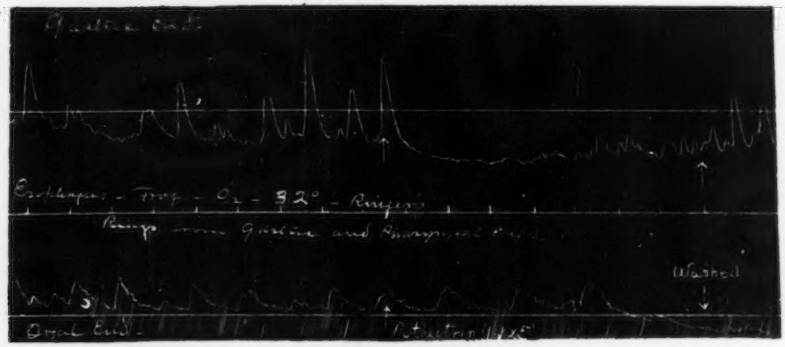

Fig. 2. Simultaneous records of the circular muscle of the opposite ends of the esophagus, showing the tardiness of reaction of the pharyngeal end as compared with that of the gastric end. Time, one minute.

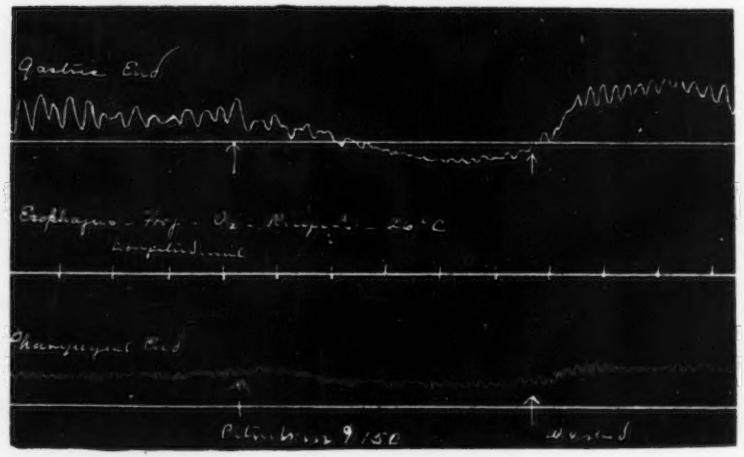

Fig. 3. Simultaneous records of the longitudinal muscle of the opposite ends of the esophagus, showing the slight effect of pituitary extract on the pharyngeal end. Time, one minute.

5. Further, endeavor was made to determine whether the depressor substance was used up in the process of depressing the tissue. In making this determination, two preparations (a and b) were suspended in separate baths. To one of these (a) pituitary extract (1:300) was applied and after the resulting depression had lasted for five minutes, the fluid was drawn off and applied to the other section (b). Depression followed in (b) as promptly as in (a) and the quantitative effect was the same.

SUMMARY

1. Pituitary extract depresses both the circular and the longitudinal musculature of the frog's esophagus.

2. In this depression, the tone, the rhythm, and the amplitude of the contractions are all affected simultaneously.

3. The depression is shown more markedly at the gastric end than at the pharyngeal.

4. Variations in the supply of oxygen, or even its total suppression, and variations in temperature do not modify the depression.

5. The depressor substance does not seem to be destroyed in the process of depressing the tissue.

6. Armour's pituitary liquid and Parke, Davis and Company's pituitrin react essentially alike on the frog's esophagus.

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